A novel gene, RSRC2, inhibits cell proliferation and affects survival in esophageal cancer patients

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Abstract. In Japan and China, esophageal cancer is common and more than 90% of esophageal cancers are squamous cell carcinoma. Esophageal squamous cell carcinoma (ESCC) shows a poor prognosis, but the mechanism of ESCC and target genes for treatment remains unclear. We searched for genes related to ESCC, and identified a novel gene, FLJ11021, which was designated *arginine/serine-rich coiled-coil 2 (RSRC2)*. We sought to determine the role of *RSRC2* in the proliferation of esophageal cell lines and to examine the relationship between *RSRC2* and clinicopathologic factors and ESCC prognosis. Expression of *RSRC2* was quantified by real-time reverse transcription polymerase chain reaction (RT-PCR) in 70 primary ESCCs and paired noncancerous esophageal mucosa. To determine the role of *RSRC2* in ESCC cell proliferation, we used vector-based transfection and small interfering RNA methods. Our results show that *RSRC2mRNA* levels in all ESCC cell lines (TE1-15, excluding TE7) were lower than those in a human esophageal squamous epithelial cell line (Het-1A). Cell proliferation of an ESCC cell line was inhibited by overexpression of *RSRC2*, while reduced expression was accompanied by tumor progression. *RSRC2* expression levels were significantly correlated with depth of invasion, lymph node metastasis, lymphatic invasion and vascular invasion. Moreover, ESCC patients with low *RSRC2mRNA* expression had significantly shorter post-operative survival time than those with high expression. *In vitro* study revealed that *RSRC2* might play a role in cell proliferation. Our study demonstrated that *RSRC2* expression may be a novel tumor suppressor of esophageal cancer cell growth and a prognostic factor in ESCC.

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Introduction

Esophageal squamous cell carcinoma (ESCC) is relatively common in Japan; worldwide, the disease is the ninth most frequent cancer, but it is the sixth most frequent cause of death from malignant tumors in Japan. ESCC exhibits poor prognosis as a result of early systemic metastasis, which occurs primarily via lymphatic vessels (1-6). Previous studies have identified prognostic factors for ESCC as tumor size, age at diagnosis, and primary site (5,6). Among these, depth of cancer invasion in the esophageal wall and lymph node metastasis are the main predictive factors for ESCC recurrence (5-7). These factors and the presence of distant metastasis are included in the pathological Tumor-Node-Metastasis (pTNM) staging for ESCC (8). The TNM classification of ESCC has been the only tool to predict recurrence and patient survival (5-7). However, the prognosis of individual ESCC, even at early stage (pT1), varies considerably, and a strategy to establish appropriate therapeutic modalities for each patient has yet to be formulated.

Molecular biologic studies have identified several genes that are involved in the carcinogenesis and/or progression of esophageal carcinoma: *TP53* (9), *CDKN2A* (10), *DEC1* (11), *DCC* (12), *DLC1* (13), *Fhit* (14), *Caveolin-1* (15), *COX-2* (16) and *MET* (17). However, the precise mechanisms that underlie the development and progression of ESCC remain unclear.

We searched for genes associated with ESCC among the Human esophageal carcinoma cDNA library (UniGene lib.6324, not available now) at the National Center for Biotechnology Information Home Page, and we obtained a list of genes associated with ESCC (Table I). Of these, we selected Hs.81648 (FLJ11021), a gene whose function was previously unknown. This gene was designated *arginine/serine-rich coiled-coil 2 (RSRC2)*. RSRC2 has arginine/serine-rich and coiled coil regions (Fig.1). *RSRC2* is predicted to act as a transcription factor, but the gene itself is poorly understood.

In this study, we analyzed the effects of *RSRC2* gene transfection and siRNA silencing of the gene on the proliferation of esophageal cancer cell lines. We also studied

Table I. Esophageal cancer-related genes.

Cluster ID	Cluster title
Hs.3463	Ribosomal protein S23 (RPS23)
Hs.81648	Hypothetical protein FLJ11021 similar to splicing factor, arginine/serine-rich4 (FLJ11021)
Hs.75789	N-myc downstream regulated (NDRG1)
Hs.65648	RNA binding motif protein 8A (RBM8A)
Hs.335928	*Homo sapiens*, similar to erythrocyte membrane protein band 4.1-like 3, clone MGC:12343 IMAGE:4044866, mRNA, complete cds
Hs.271916	ESTs, weakly similar to S41044 chromosomal protein
Hs.181304	Putative gene product (13CDNA73)
Hs.17585	KIAA0801 gene product (KIAA0801)

RSRC2mRNA expression in 70 patients with ESCC and evaluated its correlation with clinicopathologic features and postoperative survival.

Materials and methods

Cell lines and cell culture. A human esophageal squamous epithelial cell line (Het-1A) was obtained from the American Type Culture Collection (ATCC). Het-1A was maintained in serum-free medium (LHC-9; BioSource, USA) at 37˚C in a humidified 5% CO_2 incubator.

ESCC cell lines (TE1-15, excluding TE7) were obtained from the Japanese Collection of Research Bioresources (JCRB). Cultures were maintained in RPMI-1640 (Sigma) medium supplemented with 10% fetal bovine serum (FBS) (Gibco) at 37˚C in a humidified 5% CO_2 incubator.

Tissue samples. Total RNA from normal human tissues (lung, kidney, small intestine, liver, stomach, heart and spleen) was obtained from Cell Applications, Inc. RNA concentration was adjusted to 500 ng/μl using a spectrophotometer.

RNA extraction and cDNA synthesis. Total RNA was extracted from each cell line (HET-1A, TE series) using Absolutely RNA™ Miniprep kits (Stratagene, La Jolla,CA) according to the manufacturer's instructions. The reverse transcriptase reaction was carried out at 42˚C for 90 min and at 95˚C for 5 min followed by incubation at 72˚C for 15 min using 1 μg of total RNA, 0.5 mg oligo (dT) primer, and Superscript II enzyme (Gibco BRL, Gaithersburg, MD). Synthesized cDNA was adjusted to 10 ng/μl.

Quantitative reverse transcription-polymerase chain reaction (RT-PCR). Quantitative RT-PCR was performed using a LightCycler (Roche Diagnostics, Mannheim, Germany).

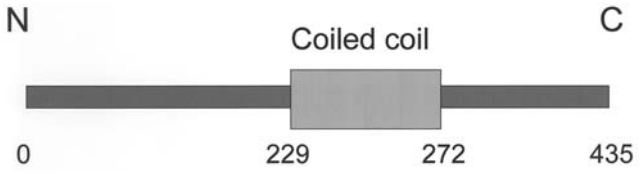

Figure 1. Structure of RSRC2 protein. RSRC2 protein consists of 434 amino acids and has a coiled coil domain.

RSRC2mRNA was amplified and quantitatively analyzed using a LightCycler-Fast Start DNA Master SYBR-Green I kit (Roche Molecular Biochemicals, Mannheim, Germany). The primer sequences for the *RSRC2* gene were as follows: forward primer, 5'-GGACAAATCCCAATCTGCTG-3'; reverse primer, 5'-GGGTTTGTGATCTTGCCATT-3' (product size: 209 base pairs). PCR protocol was as follows: initial denaturation at 95˚C for 10 min was followed by 45 cycles at 95˚C for 10 sec, annealing at 62˚C for 5 sec, and extension at 72˚C for 8 sec. PCR products were quantified based on SYBR-Green I intensity at 72˚C. RNA extracted from TE10 was used as a standard. After reverse transcription, standard cDNA was serially diluted to obtain six standard solutions for use in PCR to generate the reference curve. The relative amount of cDNA in these cell lines and tissue samples was determined by interpolation using a standard curve, and then the relative ratio of *RSRC2* to *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* expression was calculated for each sample. The primer sequences for *GAPDH* were as follows: forward primer, 5'-GAAGGTGAAGGTCGGAGTC-3'; reverse primer, 5'-GAAGATGGTGATGGGATTTC-3' (product size: 226 base pairs).

Expression vector construction. The full-length coding region of *RSRC2* was cloned into highly efficient eukaryotic expression vectors, pcDNA3.1 and pcDNA3.1/His (Invitrogen, USA). The constructed vectors (pcDNA3.1/RSRC2 and pcDNA3.1/His-RSRC2) were finally identified by restriction analysis and were verified by DNA sequencing.

Subcellular localization of RSRC2 protein by confocal microscopy. TE8 cells were transfected with pcDNA3.1/His-*RSRC2* using nucleofection technology (10^6 cells, 2 μg pcDNA3.1/His-RSRC2, Nucleofector Program U-14, Amaxa Biosystems, Germany). Transfected cells were cultured on chambered coverglass in RPMI-1640 (SIGMA) medium supplemented with 10% FBS for 48 h. Cells remaining on the coverglass were washed once with PBS and fixed with 1% paraformaldehyde for 20 min at 4˚C, and were then permeated with permeabilizing solution for 4 min. After gently washing once with PBS, cells were incubated with 0.1% Triton X-100 in PBS for 30 min on ice. Cells were incubated in 1% BSA and 4% powdered skim milk in TBS (50 mM Tris-Cl, pH 7.5, 150 mM NaCl) in order to block nonspecific antibody binding sites. Cells were then stained with primary antibody, mouse anti-His antibody (Invitrogen) (diluted 1:400 in 1% BSA in TBS), overnight at 4˚C. After washing twice with TBS, the secondary antibody, anti-mouse Alexa Fluor 488-conjugated antibody (Molecular Probes Inc.), diluted at 1:300 in 1% BSA and 1% powdered skim milk in TBS, was added. The

Table II. Relationship between *RSRC2mRNA* and clinicopathologic factors in 70 patients with esophageal squamous cell carcinoma.

Characteristic	No. of patients	*RSRC2mRNA* expression relative to GAPDH (mean ± SD)	P-value
Age at surgery (years)			
<65	42	0.207±0.108	
>65	28	0.193±0.133	NS
Gender			
Male	55	0.204±0.113	
Female	15	0.191±0.137	NS
Normal or tumor			
Normal	70	0.313±0.246	
Tumor	70	0.201±0.118	<0.01
Histologic differentiation			
Well	26	0.201±0.111	
Moderate	33	0.190±0.135	
Poor	11	0.234±0.072	NS
Tumor status			
T1	8	0.303±0.172	
T2	10	0.264±0.091	
T3	31	0.170±0.090	
T4	21	0.180±0.115	<0.01
T1 vs T2-4	8/62		<0.01
T1-2 vs T3-4	18/52		<0.01
Lymph node status			
N0	14	0.275±0.148	
N1	11	0.203±0.097	
N2	24	0.156±0.085	
N3	10	0.216±0.130	
N4	11	0.191±0.113	<0.05
Lymph node metastasis			
Negative	14	0.275±0.148	
Positive	56	0.183±0.102	<0.01
Pathologic stage			
0	3	0.299±0.131	
I	4	0.349±0.213	
II	10	0.247±0.104	
III	23	0.161±0.078	
IV	30	0.188±0.114	<0.01
Lymphatic invasion			
Negative	10	0.327±0.154	
Positive	46	0.172±0.093	<0.01
Unknown	14		
Vein invasion			
Negative	23	0.238±0.148	
Positive	33	0.173±0.090	<0.05
Unknown	14		

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SD, standard deviation; NS, not significant.

cells were incubated for 1 h at 37˚C in a dark humidified atmosphere. After washing once with TBS, cells were stained with propidium iodide (PI), diluted at 1:10000 in TBS. Coverglasses were observed by confocal laser pascal scanning microscopy (Carl Zeiss Inc.).

Cell proliferation assay. TE8 (the lowest expressor of *RSRC2mRNA* among TE series) cells were transfected with 2 μg of pcDNA3.1 or 2 μg of pcDNA3.1/RSRC2 using nucleofection technology (10^6 cells, Nucleofector Program U-14, Amaxa Biosystems, Germany) and were plated at $1x10^6$ cells/dish in 10-cm dishes under the culture conditions described above. After 24 h, positive clones were selected and harvested in medium containing G418 (400 μg/ml). The medium was changed every 2-3 days and positive clones were detected by Giemsa staining on day 7 after G418 selection.

RNA interference (RNAi) and MTT assay. Twenty-one nucleotide siRNA duplexes with symmetric two nucleotide 3'-UU overhangs were obtained from Dharmacon. TE1 (the highest expressor of *RSRC2mRNA* among the TE series) cells were transiently transfected with 100 nM of non-target siRNA control or *RSRC2* siRNA duplexes using nucleofection technology (10^6 cells, Nucleofector Program U-14) and were seeded in 96-well plates at a density of $1x10^5$ cells/100 μl. Cell proliferation was measured by the MTT method. After 3 days, 20 μl of 5 mg/ml MTT solution was added to each well and plates were incubated for 2 h at 37˚C.

Absorbance at 490 nm was determined using a SPECTRAmax 340 (Molecular Devices Corporation). Six wells were assayed for each set of conditions, and SDs were determined.

Patients and surgical specimens. For the present study, samples were obtained from 70 patients with ESCC without preoperative chemotherapy or radiation. All patients underwent radical esophagectomy with three-field lymph node dissection for ESCC at Nagoya City University Hospital between January 1996 and December 2001.

There were 55 males and 15 females and the mean age was 61.8 years (range: 43-80 years). Endoscopic examination of esophageal lesions was performed, and a histologic diagnosis of ESCC was made based on biopsy specimens obtained before surgery. Preoperative diagnostic examination, including esophagography, computed tomography, and ultrasound, were performed for the purpose of clinical staging.

The resected esophagus and lymph node were examined histologically. All samples for RT-PCR analysis were immediately frozen in liquid nitrogen and were stored at -80˚C until use. Samples were used with written consent from patients. The details of the 70 patients with ESCC are shown in Table II.

After surgery, all patients underwent laboratory examinations, such as routine peripheral blood cell counts and measurement of serum squamous cell carcinoma antigen levels every 1-6 months, and chest roentgenography, ultrasonography of the liver, computed tomography of the thorax and abdomen, and endoscopic examination of the remaining esophagus at intervals of 6-12 months.

Postoperative chemotherapy and radiotherapy were performed on 12 patients. The types of adjuvant therapies

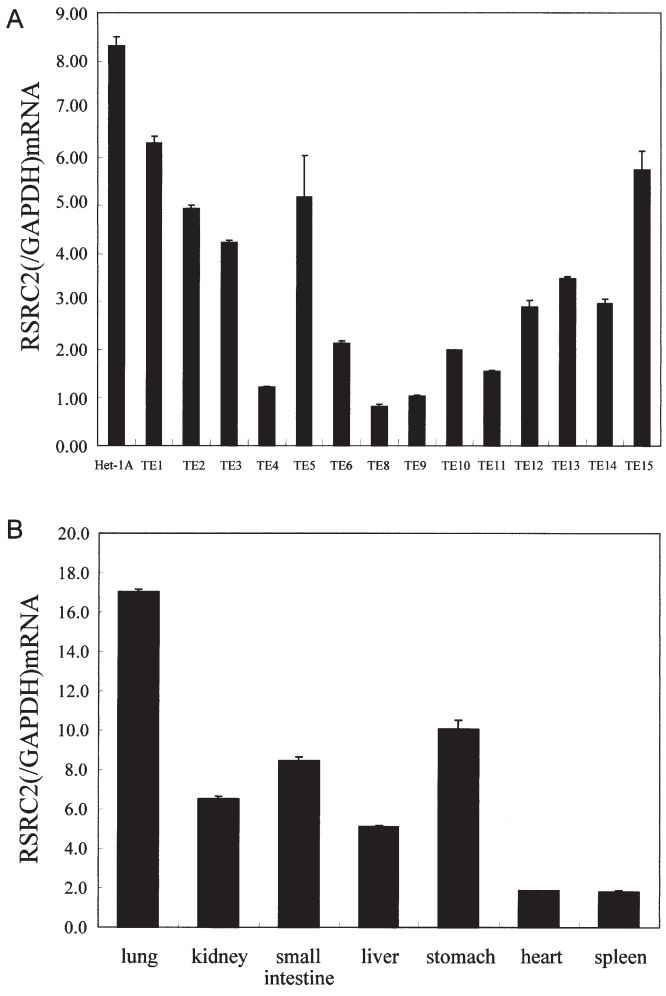

Figure 2. The expression of *RSRC2*. (A) Relative ratio of *RSRC2* to *GAPDH* expression in cell lines. *RSRC2/GAPDHmRNA* expression levels were decreased in all esophageal cancer cell lines when compared with Het-1A. Data represent the average of three independent experiments ± SD. (B) Expression of *RSRC2/GAPDHmRNA* in normal human tissues. *RSRC2* was ubiquitously expressed in several organs. Data represent the average of three independent experiments ± SD.

were as follows: postoperative chemotherapy in 1 case, radiotherapy in 8 cases, and combined chemo- and radiotherapy in 3 cases. Radiation doses ranged from 40 to 60 Gy. The chemotherapeutic agents used were 5-fluorouracil and cisplatinum. The follow-up period for survivors ranged from 1 to 55 months (median, 17.4 months). Total RNA was extracted from each sample of ESCC and its corresponding noncancerous esophageal mucosa using Absolutely RNA™ Miniprep kit, as described above. Noncancerous esophageal mucosa was taken from apparently nontumorous mucosa from a site as distant as possible from the tumor (at least 5 cm). RNA concentration was adjusted to 200 ng/μl using a spectrophotometer. cDNA synthesis and quantitative RT-PCR were performed as described above. RNA extracted from Het-1A was used as a standard. After reverse transcription, standard cDNA was serially diluted to give six standard solutions in order to generate a reference curve for PCR. The relative amounts of cDNA in these cell lines and tissue samples were measured by interpolation using the standard curve, and the relative ratio of *RSRC2* to *GAPDH* expression was calculated for each sample. All of the procedures in the present study were approved by the Ethics Committee, Nagoya City University Graduate School of Medicine.

Statistical analysis. Data are expressed as means ± standard deviation. Statistical analyses were carried out using Stat-View software (Abacus Concepts, Berkeley, CA). The significance of differences in the expression of *RSRC2mRNA* was evaluated by t-test and one-way ANOVA. The overall survival characteristics were compared using the log-rank test. Differences were considered significant at $p<0.05$.

Results

RSRC2mRNA expression in Het-1A and ESCC cell lines. In order to confirm that *RSRC2* is related to ESCC, RT-PCR was performed using ESCC cell lines. *RSRC2mRNA* expression in a human esophageal squamous epithelial cell line, Het-1A, and 14 esophageal cancer cell lines (TE1-15, excluding TE7) was examined by quantitative RT-PCR. *RSRC2mRNA* was detectable in all cell lines. *RSRC2mRNA* levels in ESCC cell lines varied; levels of *RSRC2mRNA* in all 14 cell lines were lower than those in Het-1A (Fig. 2A). *RSRC2mRNA* expression in TE4, TE8 and TE9 was particularly low.

RSRC2mRNA expression in normal human organs. We then performed quantitative RT-PCR using cDNA from various human organs. *RSRC2mRNA* was ubiquitously expressed in multiple organs and was expressed at higher levels in the lung than in the kidney, small intestine, liver, stomach, heart or spleen (Fig. 2B).

Subcellular localization of RSRC2 protein. In order to investigate the intracellular localization of RSRC2 protein, we transiently transfected a plasmid expressing His-tagged *RSRC2* (pcDNA3.1/His-RSRC2) into TE8 cells and observed the tagged protein by immunocytochemical staining. Cells transfected with pcDNA3.1/His-RSRC2 were stained with anti-His antibody and were visualized using anti-mouse secondary antibody conjugated with Alexa Fluor 488. RSRC2 protein was observed in the nuclei of TE8 cells (Fig. 3).

Overexpression of RSRC2 inhibited TE8 cell proliferation. The effects of *RSRC2* on the proliferation of esophageal cancer cells were investigated by transfecting pcDNA3.1 (empty vector) or pcDNA3.1/RSRC2 into TE8 (the lowest expressor of *RSRC2mRNA* among the TE series). The effects of the *RSRC2* expression vector were examined by quantitative RT-PCR after 3 days of G418 selection. *RSRC2mRNA* in *RSRC2*-transfected TE8 cells was overexpressed at much higher levels than in empty vector-transfected TE8 cells (Fig. 4A). Macroscopically, there were fewer colonies of the *RSRC2*-transfected TE8 cell line when compared with pcDNA3.1-transfected TE8 cells (Fig. 4B). These results indicate that overexpression of *RSRC2* inhibits the proliferation of esophageal cancer cells.

RNA interference (RNAi) and MTT assay. RNA interference was then investigated as a method to reduce *RSRC2* expression in an ESCC cell line (TE1; the highest expressor of *RSRC2*)

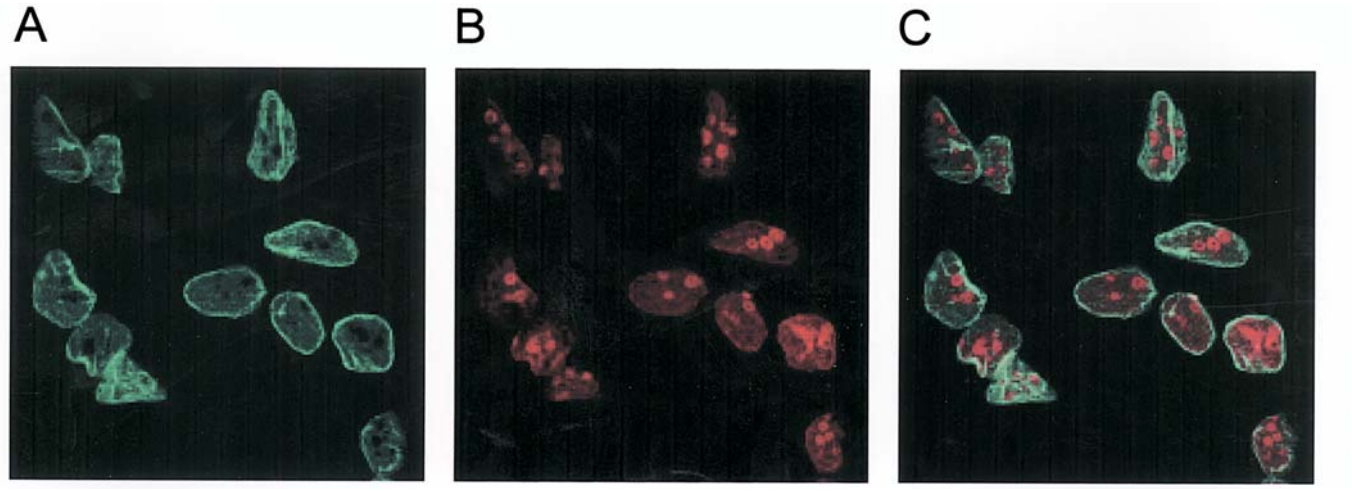

Figure 3. Subcellular localization of RSRC2 protein (x630). (A) Cells transfected with pcDNA3.1/His-RSRC2 were stained with anti-His antibody and were visualized by secondary anti-mouse antibody conjugated with Alexa Fluor 488. Green color indicates RSRC2 protein. (B) Nuclei of TE8 cells were stained with propidium iodide (PI). Nucleus were observed by the red color. (C) RSRC2 protein was found in the nuclei of TE8 cells, as revealed in merged images.

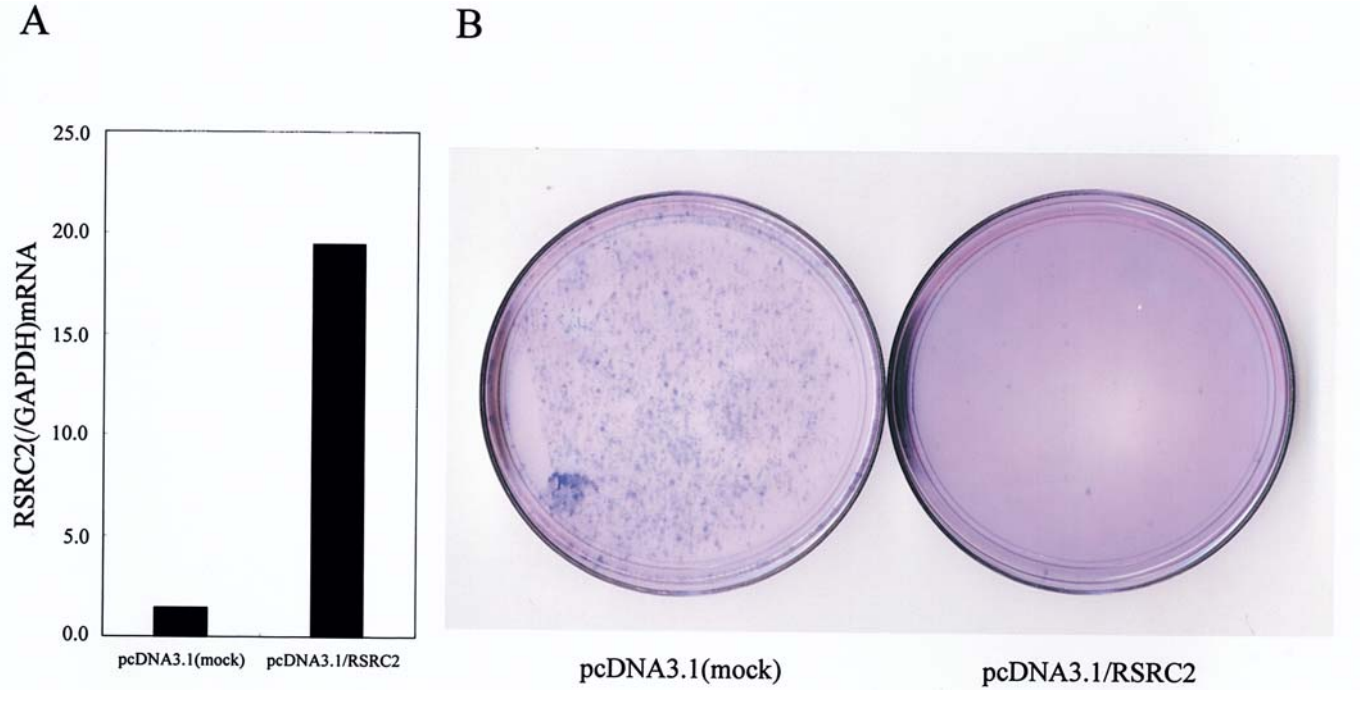

Figure 4. Overexpression of *RSRC2* inhibited TE8 cell proliferation. (A) TE8 cells were transiently transfected with pcDNA3.1 (empty vector) or pcDNA3.1/RSRC2 and were subsequently subcultured in the presence of 400 μg/ml G418. Relative expression levels of *RSRC2* were determined by quantitative RT-PCR on day 3 of G418 selection. (B) TE8 cells were transfected with pcDNA3.1 (empty vector) or pcDNA3.1/RSRC2 and were subcultured in the presence of 400 μg/ml G418. On day 7 of G418 selection, cells were stained with Giemsa and photographs of plates containing colonies were taken.

and to examine its effects on cell proliferation. On day 3 after the transfection of small interfering RNA for *RSRC2*, the expression levels of *RSRC2mRNA* were down-regulated by 90% (Fig. 5A). MTT assay confirmed that proliferation of *RSRC2* siRNA-transfected TE1 cells was mildly but significantly higher when compared with irrelevant siRNA-transfected cells (control) on day 3 (Fig. 5B).

RSRC2mRNA expression in 70 patients with ESCC. We then examined *RSRC2* expression in resected esophageal cancer tissues. Seventy primary ESCC samples and paired non-cancerous esophageal tissues were examined. *RSRC2mRNA* expression was detectable in all ESCC samples and noncancerous mucosa. *RSRC2mRNA* expression in ESCC tissue was significantly lower than that in the corresponding normal esophageal tissue ($p<0.01$, Fig. 6A). We examined the relationship between *RSRC2mRNA* expression in ESCC samples and patient clinicopathologic factors (Table II). Of the 70 ESCC samples, there were no differences in *RSRC2mRNA* levels based on age, gender or histological differentiation of ESCC. *RSRC2mRNA* expression levels in patients with ESCC varied significantly according to the

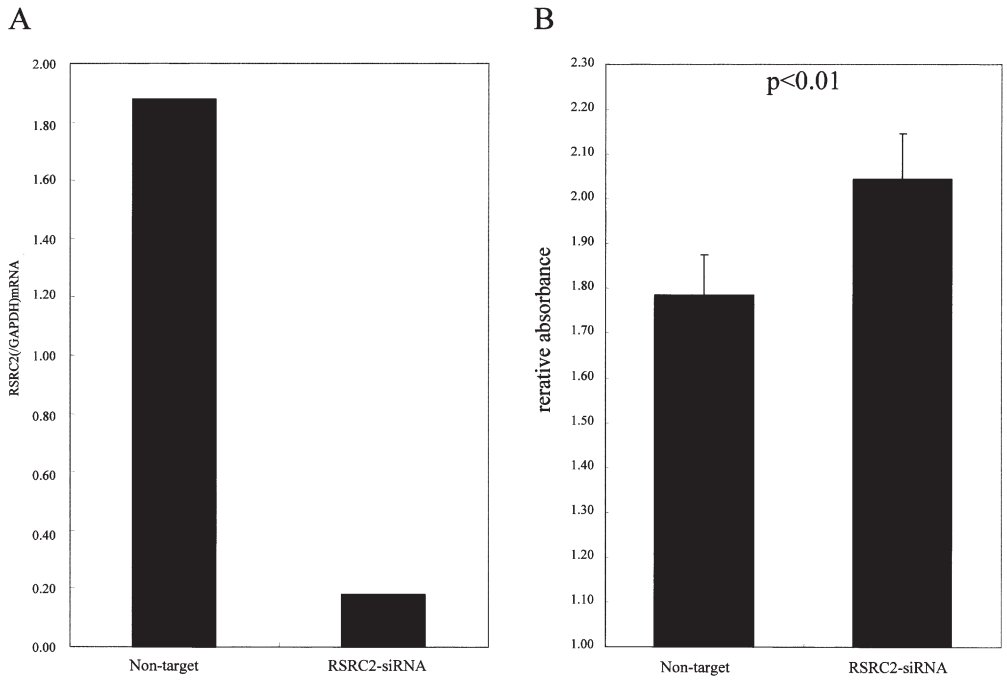

Figure 5. Effects of siRNA on *RSRC2* in TE1 cells. (A) TE1 cells were transiently transfected with 100 nM non-target siRNA control or *RSRC2* siRNA duplexes using oligofectamine (Invitrogen). The siRNA knockdown effect was examined by quantitative RT-PCR on day 3. (B) Cell proliferation of non-target siRNA- or *RSRC2* siRNA-transfected TE1 cells. TE1 cell proliferation rates were determined by MTT assay on day 3 and absorbance values were determined on a SPECTRAmax 340 (Molecular Devices Corporation) at 490 nm. Data represent the average of six independent experiments ± SD.

local tumor aggressiveness (T-status, p<0.01): *RSRC2mRNA* expression levels in locally invasive ESCC (T2-4) were significantly lower when compared to those in less invasive ESCC (T1) (p<0.01, Fig. 6B); *RSRC2mRNA* expression levels in ESCC tumors with lymph node metastasis were significantly lower when compared to those in ESCC without lymph node metastasis (p<0.01, Fig. 6C); *RSRC2mRNA* expression levels in tumors with lymphatic invasion were significantly lower when compared with those in tumors without lymphatic invasion (p<0.01); and *RSRC2mRNA* expression levels tumors with vascular invasion were significantly lower when compared to tumors without vascular invasion (p<0.05). Moreover, *RSRC2mRNA* expression levels [indicated by the ratio of *RSRC2mRNA* expression in the tumor to that in corresponding normal esophageal mucosa (T/N ratio)] in patients with ESCC varied significantly with tumor T status (p<0.01) and pathological stage (p<0.01).

Finally, we investigated the correlation between *RSRC2mRNA* expression levels and survival in patients with ESCC after tumor resection (median follow up, 17.4 months). Patients with low *RSRC2mRNA* expression levels (T/N ratio <0.6) had significantly shorter survival (14.5±1.4 months) after surgery when compared to patients with high *RSRC2mRNA* expression levels (T/N ratio >0.6) (25.1±2.5 months, p<0.05; log-rank test, Fig. 6D).

Discussion

We have demonstrated that *RSRC2* expression may serve as an additional tool to predict the malignant potential of ESCC. This newly identified gene is located at 12q24 and the gene product has 434 amino acids. However, the function of the gene product has yet to be identified. It was recently reported that FLJ11021 (*RSRC2*) might be associated with 5-fluorouracil sensitivity in gastric cancer cell lines (18); *RSRC2*-high cell lines were more sensitive to 5-fluorouracil when compared with low expression cell lines.

In this study, we found that *RSRC2* is ubiquitously expressed and that *RSRC2* protein is localized in the nucleus. *RSRC2mRNA* expression levels were decreased in many esophageal cancer cell lines and resected tumor tissues when compared with normal esophageal mucosa. In addition, cell proliferation of an esophageal cancer cell line was inhibited by overexpression of *RSRC2*. Although the mechanism of *RSRC2* downregulation is unclear, the inhibition of cell proliferation by *RSRC2* overexpression strongly suggests that *RSRC2* is related to esophageal cancer cell proliferation. Potential *RSRC2* downregulation mechanisms include LOH and hypermethylation. Surprisingly, downregulation of *RSRC2mRNA* using siRNA increased the proliferation of an esophageal cancer cell line when compared with non-target siRNA-transfected cells (control); however, the level of cell proliferation was not marked. Although the reason is not clear, this may indicate that cell growth cannot be controlled by suppressing only one gene, and it will be necessary to analyze proteins related to *RSRC2*.

Among the clinicopathologic factors examined, we observed a significant correlation between *RSRC2* expression and degree of local invasion, lymph node metastasis, lymphatic and vascular invasion, and survival of patients with ESCC. In addition to the reported correlations with clinical data, the present study suggests *RSRC2* as a candidate target gene for esophageal cancer treatment. The precise physiological function of *RSRC2* is unknown. We have previously reported

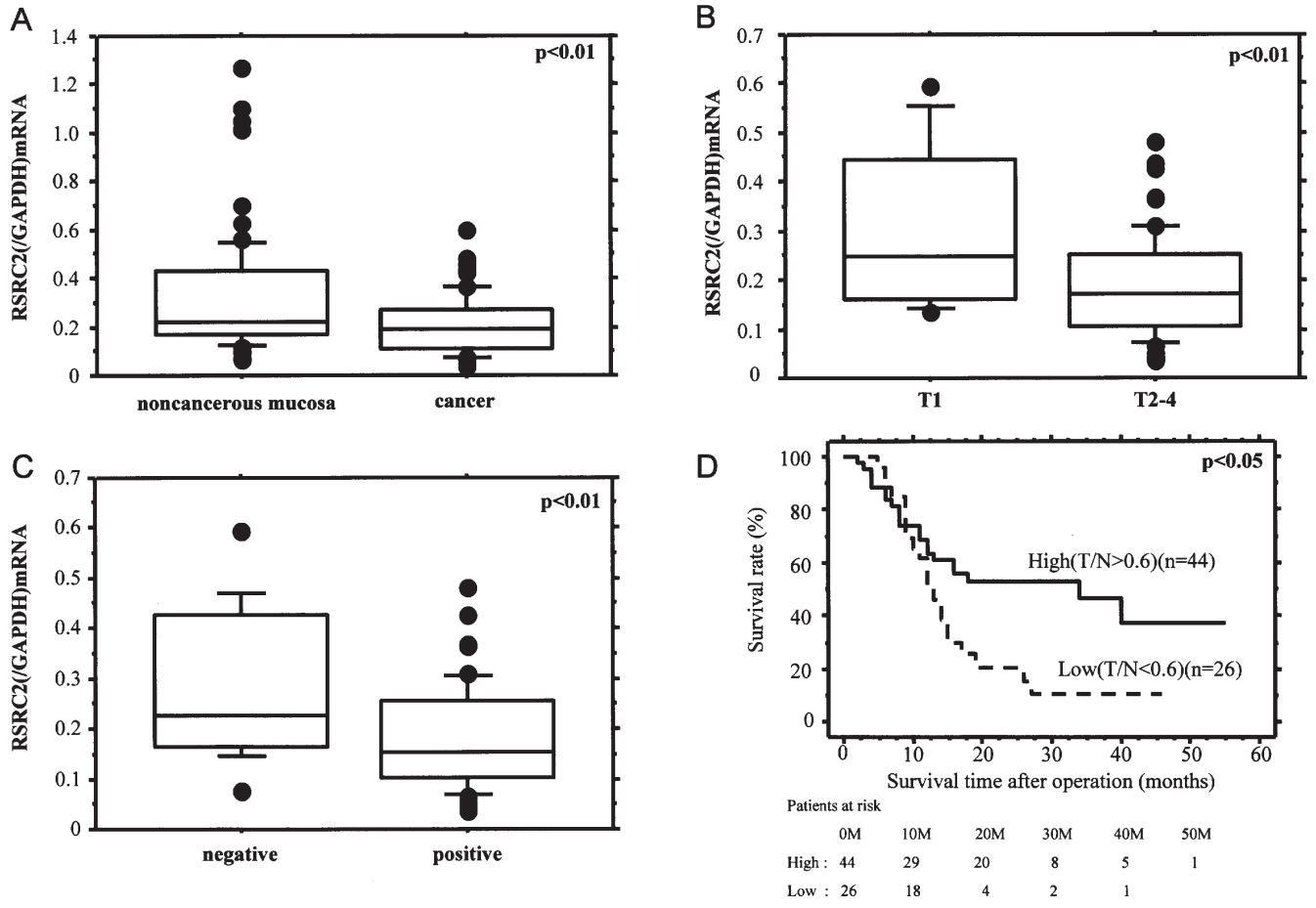

Figure 6. (A) Comparison of *RSRC2mRNA* expression between noncancerous mucosa and cancer tissue. Cancer tissue expressed lower levels of *RSRC2mRNA* than the corresponding noncancerous mucosa (n=70, p<0.01). Data represent mean ± 2SD (bars). (B) Comparison of *RSRC2mRNA* expression between early ESCC (T1) and advanced ESCC (T2-4). RSRC2mRNA expression levels in patients with advanced ESCC (T2-4) were significantly lower when compared with expression levels in patients with early ESCC (T1) (p<0.01). Data represent mean ± 2SD (bars). (C) Comparison of *RSRC2mRNA* expression between lymph node metastasis-negative and -positive ESCC. *RSRC2mRNA* expression levels in ESCC patients with lymph node metastasis were significantly lower when compared with expression levels in ESCC patients without lymph node metastasis (p<0.01). Data represent mean ± 2SD (bars). (D) Survival of patients with esophageal squamous cell carcinoma. Patients with high *RSRC2mRNA* expression [indicated by ratio of *RSRC2mRNA* expression in tumor to *RSRC2mRNA* expression in noncancerous esophageal mucosa (tumor/noncancerous) >0.6] and patients with low *RSRC2mRNA* expression [(tumor/noncancerous) <0.6]. Patients with low *RSRC2mRNA* expression had significantly shorter survival when compared to patients with high *RSRC2mRNA* expression (p<0.05; log-rank test).

genes that are related to prognosis in esophageal cancer; these include survivin (19), PTTG1 (20), PPAR-γ (21), DFF45 (22), ERCC3 (23) and PABPC1 (24). The finding that *RSRC2* is also correlated with clinical data suggests that it may be a target gene for the treatment of esophageal cancer.

In conclusion, the present study suggests that the expression of a newly identified gene, *RSRC2*, may play a significant role in esophageal cancer.

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