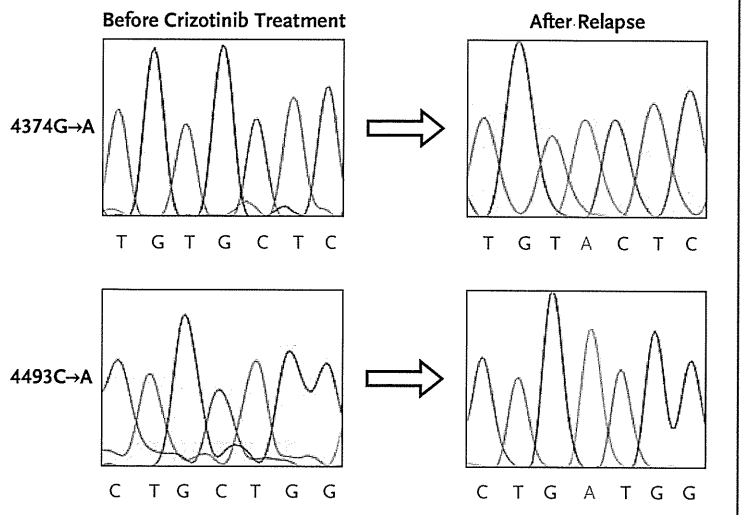

Figure 1. **Secondary Mutations within *EML4-ALK*.**
Electrophoretograms are shown for *EML4-ALK* cDNA clones prepared from sputum specimens obtained from our patient before crizotinib treatment and from pleural-effusion specimens obtained after relapse. The 4374G→A and 4493C→A mutations are present in the specimens obtained after relapse.

We next attempted to confirm these nucleotide changes by using Sanger sequencing. To rule out the possibility that the mutations had occurred in endogenous wild-type *ALK* rather than in *EML4-ALK*, we performed PCR with a forward primer targeted to *EML4* cDNA so that only the fusion cDNA would be amplified (Fig. 2 in the Supplementary Appendix). We did not detect the 4230T→C change among the 256 fusion cDNA clones derived from the patient's sputum specimens (data not shown), indicating that it was an artifact of the initial PCR or the deep-sequencing step. We did, however, readily confirm both 4374G→A and 4493C→A changes. Among 73 *EML4-ALK* cDNA clones from the patient's pleural-effusion specimens, 34 (46.6%) were positive for 4374G→A and 11 (15.1%) were positive for 4493C→A (Fig. 1). (The remaining 28 (38.4%) were negative for both point mutations.) These rates of detection are similar to those from the deep sequencing of *ALK*, indicating that wild-type *ALK* mRNA was present at a low level in lung tissue, as reported previously.[1]

The PCR analyses covered both nucleotide positions, yet none of the patient's specimens contained both mutations, indicating that each mutation occurred independently. Genomic fragments encompassing the 4374G and 4493C positions were also amplified by means of a PCR assay and were then subjected to nucleotide sequencing, which confirmed the presence of each of the two mutations in the tumor genome (Fig. 4 in the Supplementary Appendix).

The 4374G→A and 4493C→A substitutions result in cysteine→tyrosine (C→Y) and leucine→methionine (L→M) changes at the positions corresponding to amino acids 1156 and 1196, respectively, of wild-type human ALK (Fig. 2 in the Supplementary Appendix). We examined whether such amino acid changes affect the sensitivity of EML4-ALK to ALK inhibitors.

Cells of the mouse interleukin-3–dependent cell line BA/F3 that were made to individually express primary EML4-ALK and secondary mutant EML4-ALK (with the C1156Y or L1196M mutation) were exposed to ALK inhibitors. Crizotinib inhibited the growth of BA/F3 cells expressing primary EML4-ALK, in a concentration-dependent manner (Fig. 2A). In contrast, cells expressing either the C1156Y or L1196M mutant form manifested a markedly reduced sensitivity to the drug. Cells expressing the L1196M mutant form of EML4-ALK were more resistant to crizotinib than were those expressing the C1156Y mutant form (Fig. 2A, and Fig. 5 in the Supplementary Appendix).

We also examined whether cells expressing these EML4-ALK mutants are also refractory to other ALK inhibitors. A 2,4-pyrimidinediamine derivative (PDD) has a median inhibitory concentration for ALK of less than 10 nM,[11] and oral administration of PDD has been shown to eradicate lung-cancer nodules in transgenic mice with EML4-ALK expression.[4] BA/F3 cells expressing EML4-ALK with either the C1156Y or L1196M mutation were markedly less sensitive to PDD than were those expressing the primary EML4-ALK (Fig. 2A). Thus, although these mutations appear to develop during clinical treatment with crizotinib, their generation probably renders EML4-ALK resistant not only to crizotinib but also to other ALK inhibitors. In contrast to the resistance profile for crizotinib, BA/F3 cells expressing the EML4-ALK C1156Y mutant form were slightly more resistant to PDD than were those expressing the L1196M mutant form (Fig. 2A, and Fig. 6 in the Supplementary Appendix), indicating that the resistance profiles for the two mutations may be, in part, inhibitor-dependent, as was previously shown for BCR-ABL mutants.[12]

We examined tyrosine phosphorylation of

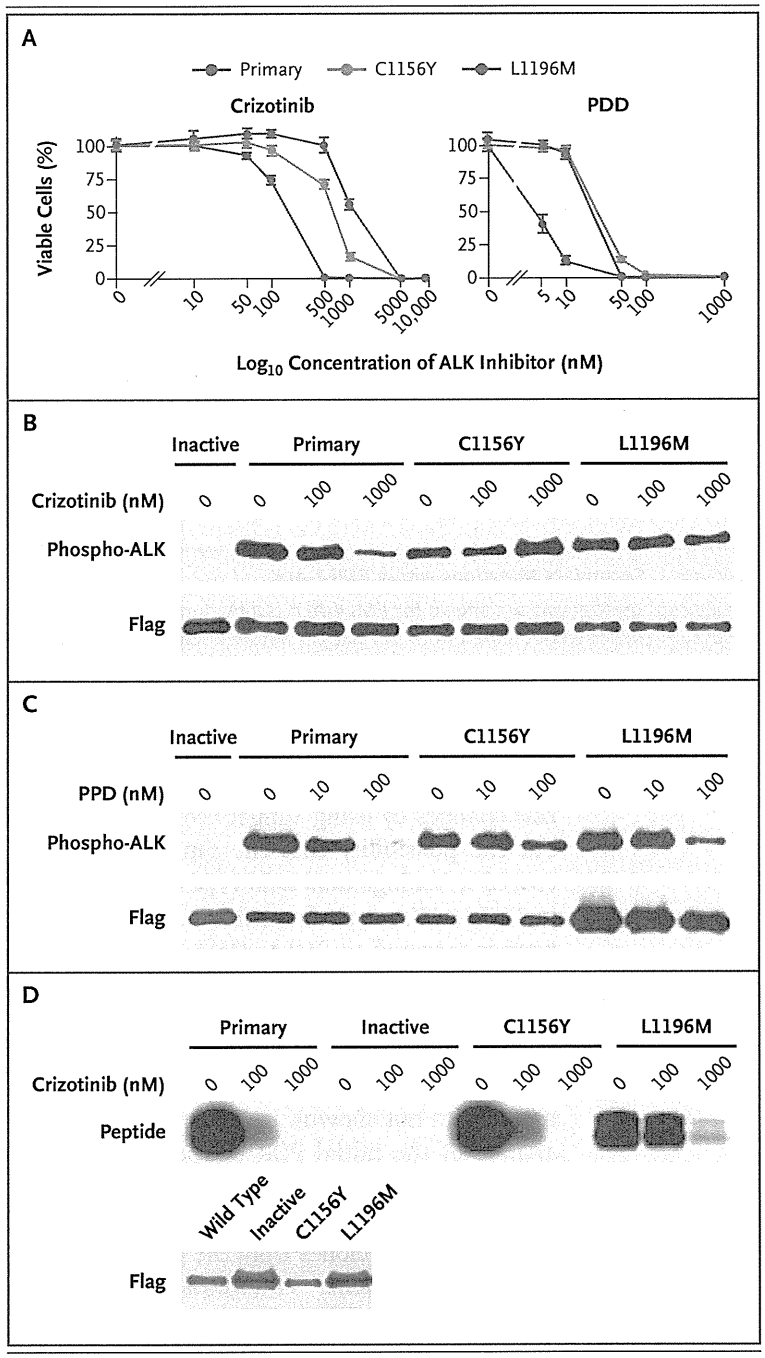

Figure 2. **Properties of EML4-ALK with Secondary Mutations.**

Panel A shows the percentage of viable BA/F3 cells expressing primary EML4-ALK, EML4-ALK with the C1156Y mutation, or EML4-ALK with the L1196M mutation, after 5×10^5 cells were incubated for 48 hours with the indicated concentration of crizotinib (left) or 2,4-pyrimidinediamine derivative (PDD) (right). Data are expressed as the mean value, from three separate experiments, for the percentage of cells expressing primary EML4-ALK after incubation in the vehicle (dimethyl sulfoxide) only. The I bars indicate standard deviations. Because primary EML4-ALK, EML4-ALK with the C1156Y mutation, and EML4-ALK with the L1196M mutation each abrogate the interleukin-3 dependence of BA/F3 cells, the assays were performed in the absence of the interleukin. Panels B and C show the effect of ALK inhibitors on EML4-ALK and its secondary mutant forms, tagged with the Flag epitope, in BA/F3 cells. Panel B shows the results of exposure to various concentrations of crizotinib for 15 hours, after which EML4-ALK was immunoprecipitated from cell lysates with antibodies against the Flag epitope and the immunoprecipitate was subjected to immunoblot analysis with the use of antibodies specific for ALK phosphorylated at the tyrosine at position 1604 (Phospho-ALK) or for the Flag epitope. Cells expressing an inactive mutant form of EML4-ALK were examined as a negative control. Panel C shows the results of a similar experiment, involving PDD instead of crizotinib. Panel D shows the results of an in vitro kinase assay for Flag-tagged EML4-ALK or its secondary mutants immunoprecipitated from BA/F3 cells with antibodies against the Flag epitope. The immunoprecipitates were incubated with [γ-^{32}P]ATP, a synthetic peptide, and various concentrations of crizotinib (top). Separate immunoprecipitate samples were subjected to immunoblot analysis with antibodies against the Flag epitope (bottom).

EML4-ALK by means of immunoblot analysis, using antibodies specific for ALK phosphorylated at the tyrosine at position 1604. The exposure of BA/F3 cells to crizotinib markedly inhibited the tyrosine phosphorylation of EML4-ALK but did not substantially affect that of the C1156Y and L1196M mutants (Fig. 2B). Exposure to PDD also inhibited the tyrosine phosphorylation of EML4-ALK, in a concentration-dependent manner, with a lesser effect on the mutants (Fig. 2C). The results of an in vitro kinase assay were consistent with these findings, showing pronounced inhibition of the enzymatic activity of primary EML4-ALK with crizotinib, whereas the effect on the C1156Y mutant was less pronounced and the effect on the L1196M mutant was much less pronounced (Fig. 2D).

Figure 3 shows the cysteine at position 1156 (C1156) and the leucine at position 1196 (L1196) of the kinase domain of ALK.[13] C1156 is positioned adjacent to the N-terminal of the predicted helix αC as well as close to the upper edge of the ATP-binding pocket. No activating mutations have been reported at this position in other tyrosine kinases in cancer specimens. L1196 of ALK corresponds to the threonine at position 315 in ABL and at position 790 in EGFR, each of which is the site of the most fre-

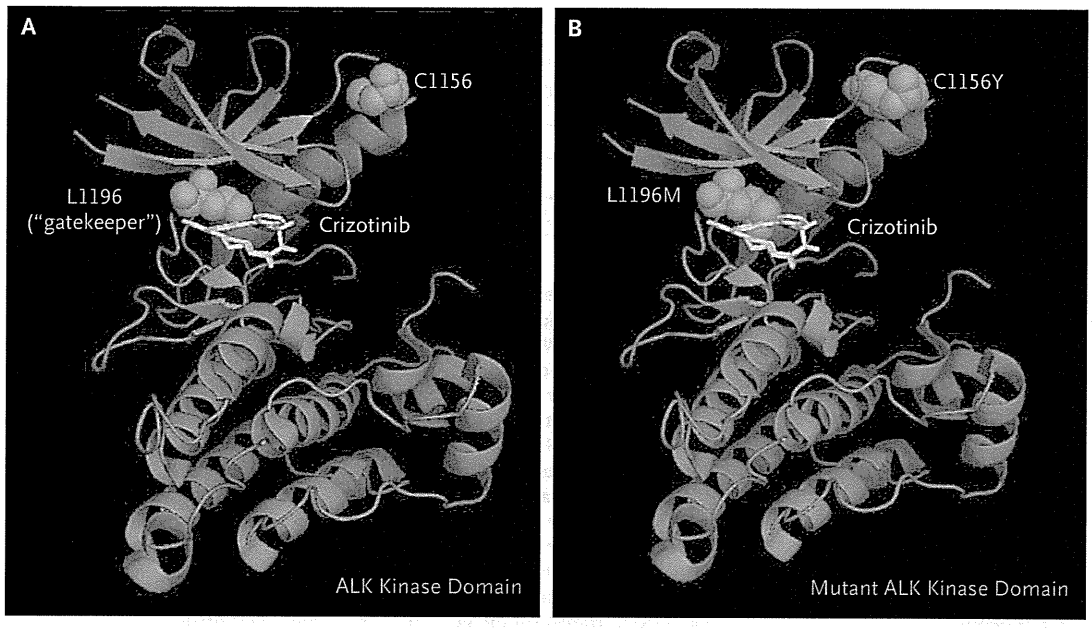

Figure 3. **Predicted Crystal Structure of the Kinase Domain of ALK.**
Panel A shows the nonmutant kinase domain and the site of binding of crizotinib. Panel B shows the EML4-ALK secondary mutations at positions 1156 and 1196. Adapted from Bossi and colleagues.[13]

quently acquired mutations that confer resistance to tyrosine kinase inhibitors in these kinases (Fig. 7 in the Supplementary Appendix).[14,15] This site is located at the bottom of the ATP-binding pocket (Fig. 3), and the presence of an amino acid with a bulky side chain at this "gatekeeper" position may interfere with the binding of many tyrosine kinase inhibitors.[7,16]

DISCUSSION

We identified two de novo mutations within the kinase domain of EML4-ALK from the tumor of a single patient that confer resistance to multiple ALK inhibitors. Given that we did not detect any *EML4-ALK* cDNA harboring both mutations, we propose that each mutation developed independently in distinct subclones of the tumor. Because we were not able to examine pleural-effusion specimens from the patient before he received crizotinib treatment, we do not know whether the resistant clones were present initially or developed secondarily, during the treatment.

Amino acid substitutions at the gatekeeper position of several tyrosine kinases have been detected in tumors treated with tyrosine kinase inhibitors (Fig. 7 in the Supplementary Appendix).[7-9,17,18] Whereas no mutations at this site have previously been reported for EML4-ALK or ALK, the effects of various artificial amino acid substitutions at the gatekeeper position of nucleophosmin (NPM)–ALK, another fusion-type "oncokinase" form of ALK, were recently examined.[19] The findings were consistent with the results of our analysis of tumor cells in vivo: the introduction of methionine at this position rendered NPM-ALK resistant to ALK inhibitors. It is therefore likely that gatekeeper alterations constitute a universal mechanism for the acquisition of tyrosine kinase–inhibitor resistance in oncogenic tyrosine kinases.

In contrast to gatekeeper substitutions, activating mutations at the position adjacent, on the N-terminal side, to the αC helix (e.g., C1156 in ALK) have not been confirmed for other tyrosine kinases in cancer specimens. Though a T→I change at the corresponding position of EGFR was described in one case of non–small-cell lung cancer, its relevance to drug sensitivity was not examined.[16] The importance of helix αC for allosteric regulation of enzymatic activity has been shown, however, for serine–threonine kinases.[20] A change at C1156 of ALK might therefore interfere allosterically with the binding of tyrosine

kinase inhibitors. Determination of the crystal structure of the ALK kinase domain with the C1156Y or L1196M mutation should shed light on these matters, as well as provide a basis for the development of next-generation ALK inhibitors that may effectively eradicate tumors harboring EML4-ALK with the acquired mutations.

Supported in part by grants from the Ministry of Health, Labor, and Welfare of Japan; the Ministry of Education, Culture, Sports, Science, and Technology of Japan; and the Japan Society for the Promotion of Science.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Y.-J. Bang and the medical staff at Seoul National University Hospital for their support in the treatment of this patient, as well as Y. Togashi and S. Hatano for technical assistance.

REFERENCES

1. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561-6.
2. Mano H. Non-solid oncogenes in solid tumors: EML4-ALK fusion genes in lung cancer. Cancer Sci 2008;99:2349-55.
3. Horn L, Pao W. EML4-ALK: honing in on a new target in non-small-cell lung cancer. J Clin Oncol 2009;27:4232-5.
4. Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci U S A 2008;105:19893-7.
5. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001;344:1031-7.
6. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
7. Shah NP, Nicoll JM, Nagar B, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2002;2:117-25.
8. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non–small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786-92.
9. Pao W, Miller VA, Politi KA, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2(3):e73.
10. Choi YL, Kaneda R, Wada T, et al. Identification of a constitutively active mutant of JAK3 by retroviral expression screening. Leuk Res 2007;31:203-9.
11. Choi YL, Takeuchi K, Soda M, et al. Identification of novel isoforms of the EML4-ALK transforming gene in non-small cell lung cancer. Cancer Res 2008;68:4971-6.
12. Branford S, Melo JV, Hughes TP. Selecting optimal second-line tyrosine kinase inhibitor therapy for chronic myeloid leukemia patients after imatinib failure: does the BCR-ABL mutation status really matter? Blood 2009;114:5426-35.
13. Bossi RT, Saccardo MB, Ardini E, et al. Crystal structures of anaplastic lymphoma kinase in complex with ATP competitive inhibitors. Biochemistry 2010;49:6813-25.
14. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. Blood 2005;105:2640-53.
15. Linardou H, Dahabreh IJ, Bafaloukos D, Kosmidis P, Murray S. Somatic EGFR mutations and efficacy of tyrosine kinase inhibitors in NSCLC. Nat Rev Clin Oncol 2009;6:352-66.
16. Carter TA, Wodicka LM, Shah NP, et al. Inhibition of drug-resistant mutants of ABL, KIT, and EGF receptor kinases. Proc Natl Acad Sci USA 2005;102:11011-6.
17. Cools J, DeAngelo DJ, Gotlib J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 2003;348:1201-14.
18. Tamborini E, Bonadiman L, Greco A, et al. A new mutation in the KIT ATP pocket causes acquired resistance to imatinib in a gastrointestinal stromal tumor patient. Gastroenterology 2004;127:294-9.
19. Lu L, Ghose AK, Quail MR, et al. ALK mutants in the kinase domain exhibit altered kinase activity and differential sensitivity to small molecule ALK inhibitors. Biochemistry 2009;48:3600-9.
20. Hindie V, Stroba A, Zhang H, et al. Structure and allosteric effects of low-molecular-weight activators on the protein kinase PDK1. Nat Chem Biol 2009;5:758-64.

Gastric Cancer (2010) 13: 245–250
DOI 10.1007/s10120-010-0566-z

Original article

Safety and efficacy of S-1 monotherapy in elderly patients with advanced gastric cancer

Takahiro Tsushima[1], Shuichi Hironaka[1,2], Narikazu Boku[1], Nozomu Machida[1], Kentaro Yamazaki[1], Hirofumi Yasui[1], Keisei Taku[1], Akira Fukutomi[1], and Yusuke Onozawa[1]

[1] Division of Gastrointestinal Oncology, Shizuoka Cancer Center, 1007 Shimonagakubo, Nagaizumi-cho, Shizuoka 411-8777, Japan
[2] Clinical Research Promotion Department, Chiba Cancer Center, Chiba, Japan

Abstract

Background. **Although S-1 is effective against advanced gastric cancer (AGC), its efficacy in elderly patients has not yet been investigated sufficiently. We assessed the efficacy and safety of S-1 monotherapy in elderly patients with AGC.**

Methods. **We conducted a retrospective review of the data of 153 patients with unresectable/recurrent gastric adenocarcinoma who received S-1 monotherapy as first-line chemotherapy at our institution. S-1 was administered orally twice daily at the dose of 40 mg/m^2, on days 1–28, every 6 weeks. We categorized the patients into three groups, the young (≤65 years old), the middle-aged (66–75 years old), and the elderly (≥76 years old); and the drug toxicity, objective responses, progression-free survivals, and overall survivals were compared among the three groups.**

Results. **The incidence of leukopenia of grade 3 or greater in the three groups was 7%, 5%, and 13%, and that of anemia was 9%, 18%, and 27%, respectively. In regard to nonhematological toxicities, the incidence of nausea of grade 3 or greater was 3%, 5%, and 13%; that of fatigue was 5%, 11%, and 20%; and that of anorexia was 5%, 6%, and 27%, respectively. As for the treatment efficacy, the objective response rates, median progression-free survivals, and overall survivals in the young, middle-aged, and elderly groups were 53%, 46%, and 33%; 7.8, 5.6, and 3.9 months; and 16.9, 17.1; and 7.7 months, respectively.**

Conclusion. **Although S-1 monotherapy showed moderate efficacy in elderly (≥76 years) patients with AGC, patients in this age group showed higher incidences of severe toxicities than the younger patients.**

Key words S-1 · Elderly · Gastric Cancer · Safety · Efficacy

Introduction

Gastric cancer is the second leading cause of death from malignant disease in the world [1, 2]. In Japan, gastric cancer is the most frequently encountered malignancy and the second leading cause of cancer-related death [3]. The prognosis of unresectable or recurrent tumors is very poor: the median survival time is about 4 months with best supportive care [4–6]. Although several randomized trials of treatments for advanced gastric cancer were conducted during the 1990s, with anthracyclines, mitomycin C, 5-fluorouracil (5-FU), methotrexate, and cisplatin [7–15], no standard treatment for advanced gastric cancer was established.

S-1 is an oral fluoropyrimidine, consisting of tegafur (a prodrug of fluorouracil), 5-chloro-2, 4-dihydropyrimidine (CDHP), and potassium oxonate. CDHP is an inhibitor of dihydropyrimidine dehydrogenase (DPD), which is the rate-limiting enzyme for the degradation of fluorouracil [16]. Three randomized controlled trials of S-1 monotherapy have been reported from Japan. One was the Japan Clinical Oncology Group (JCOG) 9912 trial, which showed the noninferiority of S-1 to continuous infusion of 5-FU, adopted as the reference arm for patients with unresectable or recurrent gastric cancer, based on the result of the JCOG9205 trial [15, 17]. The second trial was the S-1 plus cisplatin versus S-1 in RCT in the treatment for stomach cancer (SPIRITS) trial, conducted in 2007, which showed the superiority of S-1 plus cisplatin to S-1 alone in patients with advanced gastric cancer [18]. The third trial was the randomized phase III study of irinotecan plus S-1 (IRIS) versus S-1 alone as first-line treatment for advanced gastric cancer (GC0301/TOP-002), which did not demonstrate the superiority of S-1 plus irinotecan (CPT-11) to S-1 alone [19]. From the results of these three phase III trials, S-1 plus cisplatin came to be recognized as the standard of care for patients with advanced gastric cancer in Japan, while S-1 monotherapy was a community standard until 2007.

In recent years, the percentage of elderly people in the general population in Japan has increased remarkably, to more than 20%, owing to the prolonged lifespan of the

Offprint requests to: T. Tsushima
Received: April 8, 2010 / Accepted: July 6, 2010

Japanese. Considering this social background, chemotherapy for elderly cancer patients is an important issue that must be addressed. However, because gastric cancer patients who were more than 75 years old were not included in the three aforementioned Japanese phase III trials, elderly patients are generally administered monotherapy with S-1, which is not as intensive as S-1 plus cisplatin. However, the efficacy and toxicity of S-1 monotherapy in elderly patients has not yet been clarified.

In this study, we assessed the safety and efficacy of S-1 monotherapy as a function of the age of patients with advanced gastric cancer.

Subjects, materials, and methods

Patients

The subjects were patients with unresectable or recurrent gastric cancer who received S-1 monotherapy at our hospital. The patient selection criteria were as follows: Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2; histologically proven adenocarcinoma; no previous history of chemotherapy; adequate oral intake; adequate bone marrow, renal, and hepatic functions (defined as an absolute neutrophil count of ≥1500/μl, hemoglobin of ≥8.0 g/dl, serum creatinine of ≤1.5 mg/dl, serum transaminase levels less than threefold the upper limit of normal); and no concomitant malignancy. The presence of measurable lesions was not mandatory.

We categorized the patients into three groups, as follows; the young group (less than 66 years old), the middle-aged group (66 years or older, but not older than 75 years), and the elderly (more than 75 years old).

Treatment dose and schedule

S-1 was administered orally twice daily at the dose of 40 mg/m^2 from day 1 to day 28, followed by 14 days' rest, and this regimen was repeated every 42 days until disease progression, the appearance of unacceptable toxicities, or the patient's refusal to continue treatment. The dosage of S-1 was determined according to the body surface area (BSA), as follows: BSA less than 1.25 m^2, 40 mg bid; BSA 1.25 to 1.5 m^2, 50 mg bid; BSA more than 1.5 m^2, 60 mg bid. We suspended treatment during the cycle or delayed the treatment cycle until nonhematological toxicities recovered to grade 1 or lower, the neutrophil count was 1500/μl, and the platelet count was 7.5×10^4/μl. The dose of S-1 was reduced by 20% (level 1) in the event of any of the following occurrences during the previous cycle: grade 4 decrease in the leukocyte count, hemoglobin, or platelet count; and/or grade 3 or higher nonhematological toxicities. If these toxicities appeared again at the reduced dose, an additional reduction of the dose of S-1 by 20% (level 2) was made. The treatment schedule of 2 weeks' administration every 3 weeks was permitted if severe adverse events were seen after the second week in each course. A dose reduction of S-1 by one level at the initiation of the therapy was also permitted considering the patient's age, PS, and organ functions.

Response and toxicity evaluation

We obtained all the clinical data from the medical records retrospectively. We repeated physical examinations and laboratory tests at least once every 2 weeks. Objective response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0, and toxicity was evaluated based on the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

Statistical analysis

Overall survival (OS) was defined as the period from the date of the first administration of S-1 to the date of death from any cause or the last date on which the patient was confirmed to be alive. Progression-free survival (PFS) was defined as the period from the date of the first administration of S-1 to the date of confirmation of tumor progression by imaging, or the date of symptomatic deterioration by clinical judgment, or the last date on which the patient was confirmed to be alive without disease progression. Patients who had only the one noncurative factor of positive peritoneal washing cytology were excluded from the PFS and OS analyses, because it was suggested that these patients would survive longer than patients with other noncurative factors; however these patients' toxicities were assessed. Patients who did not have target lesions were also excluded from the response rate (RR) analysis. The survival curves were calculated by the Kaplan-Meier method, using StatView, version 5.0 (Abacus Concepts, Berkeley, CA, USA). Written informed consent was obtained from each of the patients prior to their starting the chemotherapy.

Results

Patient characteristics

A total of 165 patients received S-1 monotherapy between September 2002 and October 2007. Of these, 12 patients were excluded, for the following reasons: hepatic function disorder (5 patients), concomitant malignancy (3 patients), severe anemia (2 patients), renal failure (1 patient), and massive pleural effusion and/or ascites (1 patient).

Table 1. Patient characteristics at baseline

		Young ($n = 76$)	Middle-aged ($n = 62$)	Elderly ($n = 15$)	P value
Age (years)	Median	59.5	70	77	<0.0001
	(range)	(34–65)	(66–75)	(76–80)	
Sex	Male	52	49	10	0.33
	Female	24	13	5	
PS	0	43	26	4	0.02
	1	32	35	8	
	2	1	1	3	
Tumor status	Unresectable	59	54	14	0.18
	Recurrent	17	8	1	
CCr (ml/min)[a]	Median	88.3	65.4	59.9	<0.0001
	(range)	(35.5–143.7)	(35.9–104.9)	(41.3–93.9)	
Macroscopic type	1	0	2	2	0.16
	2	19	16	7	
	3	35	32	2	
	4	20	9	4	
	Unknown	2	3	0	
Histological type	Intestinal	15	26	8	0.01
	Diffuse	60	32	7	
	Unknown	1	4	0	
No. of metastatic sites	1	54	28	5	0.003
	2	18	31	7	
	≥3	4	3	3	
Target lesions	+	32	35	12	0.02
	–	44	27	3	
Noncurative factors	Only CY1	20	12	1	0.21
	Others	56	50	14	

The P values were determined using the Kruskal-Wallis test
PS, performance status; CCr, creatinine clearance; CY1, positive peritoneal washing cytology
[a] Cockcroft-Gault equation

The baseline characteristics of the patients in the three groups are shown in Table 1. The median age was 59.5 years (range, 34 to 65 years) in the young group, 70 years (range, 66 to 75 years) in the middle-aged group, and 77 years (range, 76 to 80 years) in the elderly group. The percentage of patients with PS 2 was higher (20%) in the elderly group than in the other two groups. The median creatinine clearance (calculated by the Cockcroft-Gault equation) was 88.3 ml/min in the young group, 65.4 ml/min in the middle-aged group, and 59.9 ml/min in the elderly group. There were 32 (42%), 35 (56%), and 12 (80%) patients with target lesions, and 20 (26%), 12 (19%), and 1 (6%) patients with positive peritoneal washing cytology as the only noncurative factor in the young, middle-aged, and elderly groups, respectively.

Exposure to treatment

The median number of treatment cycles was 5.5 (range, 1 to 28) in the young group, 5 (range, 1 to 18) in the middle-aged group, and 3 (range, 1 to 13) in the elderly group. Dose reduction of S-1 was required in some patients in all three groups: in 12 patients (16%) in the young group, 14 patients (23%) in the middle-aged group, and 8 patients (53%) in the elderly group. Delay of the subsequent treatment cycle was also necessitated in some patients in all three groups: in 23 patients (30%) in the young group, 26 patients (42%) in the middle-aged group, and 5 patients (33%) in the elderly group.

The median relative dose intensity (RDI) per patient in the elderly group was only 75.8%, whereas the corresponding values in the young and middle-aged groups were 99.5% and 96.3%. In 7 (47%) of the 15 patients in the elderly group, S-1 was administered at a reduced dose from the start, and in 8 patients in the elderly group (53%), the dose of S-1 was reduced due to the appearance of toxicity during the treatment courses, and 3 of these 8 patients needed additional dose reduction because of the development of severe adverse events.

The reasons for treatment discontinuation are shown in Table 2. The most frequent reason in all three groups was disease progression. While two patients in the young group required treatment discontinuation because of the development of adverse events (grade 3 pneumonitis in one, and grade 2 skin rash in the other), none of the patients in the elderly group required treatment

Table 2. Reasons for treatment discontinuation

	Young (n = 76)	Middle-aged (n = 62)	Elderly (n = 15)
S-1 discontinuation	74 (97%)	62 (100%)	15 (100%)
Disease progression	60	52	13
Adverse events	2	0	0
Patient's refusal	0	3	0
Lost to follow-up	1	1	1

Table 3. Adverse events

	Young (n = 76)				Middle-aged (n = 62)				Elderly (n = 15)				
	G1/2	G3	G4	≥G3 (%)	G1/2	G3	G4	≥G3 (%)	G1/2	G3	G4	≥G3 (%)	P value
Hematological													
Leukopenia	41	5	0	7	36	3	0	5	3	2	0	13	0.28
Neutropenia	26	10	1	14	25	5	0	8	2	2	0	13	0.45
Anemia	63	7	0	9	51	10	1	18	9	4	0	27	0.08
Thrombocytopenia	21	1	0	1	15	1	0	2	6	0	0	0	0.59
Nonhematological													
Nausea	28	2	0	3	16	3	0	5	7	2	0	13	0.08
Vomiting	16	0	0	0	9	0	0	0	5	0	0	0	0.24
Anorexia	40	4	0	5	34	4	0	6	8	4	0	27	0.07
Diarrhea	29	0	0	0	16	0	0	0	5	0	0	0	0.31
Mucositis	25	1	0	1	22	4	0	6	6	0	0	0	0.53
Fatigue	19	4	0	5	21	7	0	11	8	3	0	20	0.004
Febrile neutropenia	—	0	0	0	—	0	0	0	—	0	0	0	
Death within 30 days				0				0				1	

The P values were determined using the Kruskal-Wallis test

discontinuation because of adverse events or the patient's refusal.

Adverse events

Table 3 shows the adverse events until 30 days from the last administration of S-1 or the day of initiation of second-line treatment, whichever was earlier. The incidences of grade 3/4 decreases in the leukocyte count and serum hemoglobin were 7% and 9% in the young group, 5% and 18% in the middle-aged group, and 13% and 27% in the elderly group, respectively. One patient in the middle-aged group showed a grade 4 decrease in serum hemoglobin, and one patient in the young group showed grade 4 neutropenia. In regard to the nonhematological toxicities, the incidences of grade 3/4 nausea, anorexia, and fatigue seemed to be higher in the elderly group than in the other two groups. Thus, it would appear that patients in the elderly group experienced more severe hematological and nonhematological toxicities than those in the young and middle-aged groups, while the incidences of toxicities were similar in the young and middle-aged groups. One patient in the elderly group died within 30 days from the last administration of S-1. He was 80 years old, with PS 2, and the estimated creatinine clearance was 56 ml/min at the baseline. He received S-1 administration at a reduced dose even from the first cycle because of his advanced age and anorexia caused by primary cancer. When he visited our hospital on day 17, he was found to have grade 2 mucositis. On day 23, he was admitted to another hospital because of severe anorexia and fatigue, and received some infusion therapy. However, he died on day 30 after the last administration of S-1. The attending physician judged that the cause of death was disease progression.

Response and survival

Among the patients with target lesions, the RR was 53% (17/32) in the young group, 46% (16/35) in the middle-aged group, and 33% (4/12) in the elderly group. Two patients (6%) in the middle-aged group showed a complete response (CR).

The data of 56 patients in the young group, 50 in the middle-aged group, and 14 in the elderly group were analyzed in the calculations of the PFS and OS. The median PFS values in the young group, middle-aged group, and elderly group were 7.8 months, 5.6 months, and 3.9 months (Fig. 1), and the median overall survivals

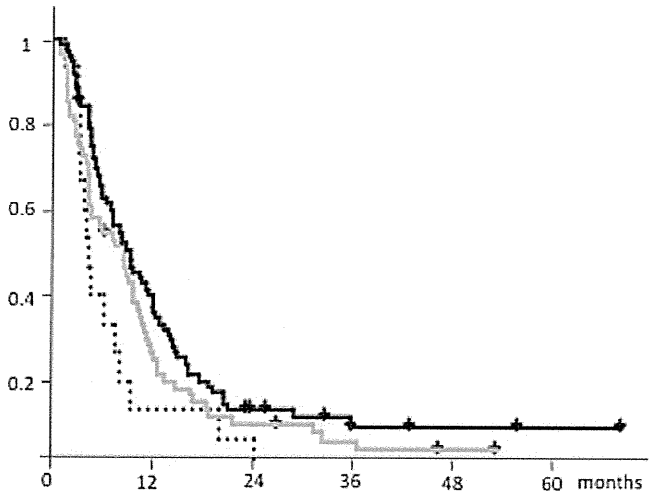

Fig. 1. The median progression-free survival (PFS) was 7.8 months in the young group (*solid line*), 5.6 months in the middle-aged group (*faint line*), and 3.9 months in the elderly group (*dotted line*)

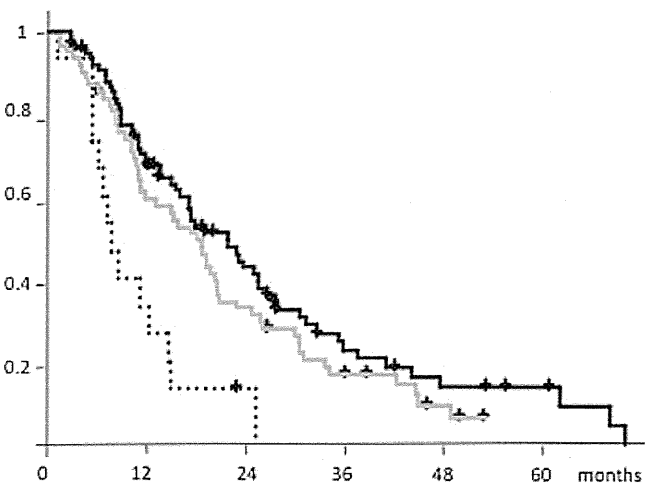

Fig. 2. The median OS was 16.9 months in the young group (*solid line*), 17.1 months in the middle-aged group (*faint line*), and 7.7 months in the elderly group (*dotted line*)

in the three groups were 16.9 months, 17.1 months, and 7.7 months, respectively (Fig. 2). It would seem that the treatment efficacy in the elderly group was inferior to that in the other two groups, while the young and middle-aged groups showed similar treatment efficacy.

Discussion

It still remains under debate whether standard chemotherapies established by pivotal phase III trials might also be applicable to elderly patients with advanced gastric cancer [20–23]. Lee et al. [24] conducted a randomized phase II study comparing capecitabine and S-1 in patients older than 65 years, and showed satisfactory efficacy of S-1 (RR, 29%; median time to progression, 4.2 months; median OS, 8.1 months). In Japan, Koizumi et al. [25] conducted a phase II study of S-1 in patients older than 75 years and demonstrated a RR of 21%, median PFS of 3.9 months, and median OS of 15.7 months. Similar results were obtained in the elderly group in the present study. Because these results are consistent with those of the previous phase III studies in Japan [17–19] (RR of about 30% and PFS of about 4 months), it is considered that S-1 monotherapy may be effective in elderly patients with gastric cancer.

However, elderly cancer patients often have comorbidities and age-related physiological problems, such as organ dysfunction. The kidney is a very common route for the excretion of drugs; however, it is reported that the glomerular filtration rate generally decreases by approximately 0.75 ml/min per year after the age of 40, on average [26]. In several pharmacokinetic studies of chemotherapeutic drugs, such as paclitaxel, vinorelbine, etoposide, cisplatin, and doxorubicin, an age-related decrease in creatinine clearance has been reported [23].

Lee et al. [24] and Koizumi et al. [25] reported the following incidences of grade 3/4 toxicities: decrease in serum hemoglobin, 9%–14.3%; anorexia, 9.5%–12%; and nausea, 4.8%–6%. These data are similar to those in the middle-aged group in the present study (decrease in serum hemoglobin, 18%; anorexia, 6%; nausea, 5%). In the elderly group in the present study, the incidences of severe toxicities (decrease in serum hemoglobin, 27%; anorexia, 27%; nausea, 13%) were higher than those reported from the previous trials, despite about half of our elderly patients having received S-1 at a reduced dose from the first administration. The conditions of patients in daily clinical practice are generally worse than those in patients participating in clinical trials. Actually, in the present study, the median creatinine clearance, estimated by the Cockcroft-Gault equation, was lower in the elderly group (59.9 ml/min) than the values in the middle-aged (65.4 ml/min) and younger (88.3 ml/min) groups. It is known that the clearance of CDHP is reduced by renal dysfunction, resulting in a high blood concentration of 5-FU due to decreased DPD activity [27, 28]. In a post-marketing survey of S-1, it was reported that the incidence of toxicities was greater in patients with renal dysfunction than in those with normal renal function [29]. Thus, it is considered that renal dysfunction is the main reason for the high incidence of severe S-1 toxicities in elderly patients. Therefore, careful evaluation of the renal function prior to the initiation of S-1 monotherapy is strongly recommended.

In conclusion, in the present study, although S-1 monotherapy exhibited moderate efficacy in elderly

patients (≥76 years of age) with advanced gastric cancer, this subject population is at a higher risk of severe toxicities than the other two age groups (66–75 years old and younger) examined in this study. Careful monitoring of renal function and toxicities during treatment is recommended, especially in elderly patients.

References

1. Munoz N, Franceschi S. Epidemiology of gastric cancer and perspectives for prevention. Salud Publica Mex 1997;39:318–30.
2. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 2006;24:2137–50.
3. Inoue M, Tsugane S. Epidemiology of gastric cancer in Japan. Postgrad Med J 2005;81:419–24.
4. Murad AM, Santiago FF, Petroianu A, Rocha PR, Rodrigues MA, Rausch M. Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. Cancer 1993;72:37–41.
5. Glimelius B, Hoffman K, Haglund U, Nyren O, Sjoden PO. Initial or delayed chemotherapy with best supportive care in advanced gastric cancer. Ann Oncol 1994;5:189–90.
6. Pyrhonen S, Kuitunen T, Nyandoto P, Kouri M. Randomised comparison of fluorouracil, epidoxorubicin and methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. Br J Cancer 1995; 71:587–91.
7. Cullinan SA, Moertel CG, Fleming TR, Rubin JR, Krook JE, Everson LK, et al. A comparison of three chemotherapeutic regimens in the treatment of advanced pancreatic and gastric carcinoma. Fluorouracil versus fluorouracil and doxorubicin versus fluorouracil, doxorubicin, and mitomycin. JAMA 1985;253: 2061–7.
8. Wils JA, Klein HO, Wagener DJ, Bleiberg H, Reis H, Korsten F, et al. Sequential high-dose methotrexate and fluorouracil combined with doxorubicin–a step ahead in the treatment of advanced gastric cancer: a trial of the European Organization for Research and Treatment of Cancer Gastrointestinal Tract Cooperative Group. J Clin Oncol 1991;9:827–31.
9. Kelsen D, Atiq OT, Saltz L, Niedzwiecki D, Ginn D, Chapman D, et al. FAMTX versus etoposide, doxorubicin, and cisplatin: a random assignment trial in gastric cancer. J Clin Oncol 1992; 10:541–8.
10. Cocconi G, Bella M, Zironi S, Algeri R, Di Costanzo F, De Lisi V, et al. Fluorouracil, doxorubicin, and mitomycin combination versus PELF chemotherapy in advanced gastric cancer: a prospective randomized trial of the Italian Oncology Group for Clinical Research. J Clin Oncol 1994;12:2687–93.
11. Webb A, Cunningham D, Scarffe JH, Harper P, Norman A, Joffe JK, et al. Randomized trial comparing epirubicin, cisplatin, and fluorouracil versus fluorouracil, doxorubicin, and methotrexate in advanced esophagogastric cancer. J Clin Oncol 1997;15:261–7.
12. Vanhoefer U, Rougier P, Wilke H, Ducreux MP, Lacave AJ, Van Cutsem E, et al. Final results of a randomized phase III trial of sequential high-dose methotrexate, fluorouracil, and doxorubicin versus etoposide, leucovorin, and fluorouracil versus infusional fluorouracil and cisplatin in advanced gastric cancer: a trial of the European Organization for Research and Treatment of Cancer Gastrointestinal Tract Cancer Cooperative Group. J Clin Oncol 2000;18:2648–57.
13. Kim NK, Park YS, Heo DS, Suh C, Kim SY, Park KC, et al. A phase III randomized study of 5-fluorouracil and cisplatin versus 5-fluorouracil, doxorubicin, and mitomycin C versus 5-fluorouracil alone in the treatment of advanced gastric cancer. Cancer 1993;71:3813–8.
14. Cullinan SA, Moertel CG, Wieand HS, O'Connel MJ, Poon MA, Krook JE, et al. Controlled evaluation of three drug combination regimen versus fluorouracil alone in the therapy of advanced gastric cancer. J Clin Oncol 1994;12:412–6.
15. Ohtsu A, Shimada Y, Shirao K, Boku N, Hyodo I, Saito H, et al. Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: The Japan Clinical Oncology Group Study (JCOG9205). J Clin Oncol 2003;21:54–9.
16. Shirasaka T, Shimamoto Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K, et al. Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 1996;7:548–57.
17. Boku N, Yamamoto S, Fukuda H, Shirao K, Doi T, Sawaki A, et al. Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study. Lancet Oncol 2009;10:1063–9.
18. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, et al. S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol 2007;9:215–21.
19. Imamura H, Ishii H, Tsuburaya A, Hatake K, Imamoto H, Esaki T, et al. Randomized phase III study of irinotecan plus S-1 (IRIS) versus S-1 alone as first-line treatment for advanced gastric cancer (GC 0301/TOP-002). ASCO-GI 2008;Abstract no. 5.
20. Lichtman SM, Wildiers H, Chatelut E, Steer C, Budman D, Morrison VA, et al. International Society Of Geriatric Oncology Chemotherapy Taskforce: evaluation of chemotherapy in older patients — an analysis of the medical literature. J Clin Oncol 2007;25:1832–43.
21. Repetto L, Balducci L. A case of geriatric oncology. Lancet Oncol 2002;3:289–97.
22. Lichtman SM, Boparai MK. Anticancer drug therapy in the older cancer patient: pharmacology and polypharmacy. Curr Treat Options Oncol 2008;9:191–203.
23. Hurria A, Lichtman SM. Clinical pharmacology of cancer therapies in older adults. Br J Cancer 2008;98:517–22.
24. Lee JL, Kang YK, Kang HJ, Lee KH, Zang DY, Ryoo BY, et al. A randomised multicentre phase II trial of capecitabine vs S-1 as first-line treatment in elderly patients with metastatic or recurrent unresectable gastric cancer. Br J Cancer 2008;99:584–90.
25. Koizumi W, Akiya T, Sato A, Sakuyama T, Sasaki E, Tomidokoro T, et al. Phase II study of S-1 as first-line treatment for elderly patients over 75 years of age with advanced gastric cancer: the Tokyo Cooperative Oncology Group Study. Cancer Chemother Pharmacol 2010;65:1093–9.
26. Lindeman RD, Tobin J, Shock NW. Longitudinal studies of decline in renal function with age. J Am Geriatr Soc 1985;33:278–85.
27. Tatsumi K, Fukushima M, Shirasaka T, Fujii S. Inhibitory effects of pyrimidine, barbituric acid and pyridine derivatives on 5-fluorouracil degradation in rat liver extracts. Jpn J Cancer Res 1987;78:748–55.
28. Hirata K, Horikoshi N, Aiba K, Okazaki M, Denno R, Sasaki K, et al. Pharmacokinetic study of S-1, a novel oral fluorouracil antitumor drug. Clin Cancer Res 1999;5:2000–5.
29. Nagashima F, Ohtsu A, Yoshida S, Ito K. Japanese nationwide post-marketing survey of S-1 in patients with advanced gastric cancer. Gastric Cancer 2005;8:6–11.

VOLUME 28 · NUMBER 33 · NOVEMBER 20 2010

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Association of DNA Repair Gene Polymorphisms With Response to Platinum-Based Doublet Chemotherapy in Patients With Non–Small-Cell Lung Cancer

Kouya Shiraishi, Takashi Kohno, Chiharu Tanai, Yasushi Goto, Aya Kuchiba, Seiichiro Yamamoto, Koji Tsuta, Hiroshi Nokihara, Noboru Yamamoto, Ikuo Sekine, Yuichiro Ohe, Tomohide Tamura, Jun Yokota, and Hideo Kunitoh

From the National Cancer Center Research Institute; National Cancer Center Hospital; and the Center for Cancer Control and Information Services, National Cancer Center, Tokyo, Japan.

Submitted May 17, 2010; accepted July 30, 2010; published online ahead of print at www.jco.org on October 12, 2010.

Supported in part by Grant-in-Aid No. KAKENHI 20014031 from the Ministry of Education, Culture, Sports, Science and Technology for Scientific Research on Priority Areas, and Nos. 19S-1 and 19-9 from the Ministry of Health, Labor and Welfare for Cancer Research and by the 3rd-term Comprehensive 10-Year Strategy for Cancer Control. K.S. received a Research Resident Fellowship from the Foundation for Promotion of Cancer Research in Japan.

Authors' disclosures of potential conflicts of interests and author contributions are found at the end of this article.

Corresponding author: Takashi Kohno, PhD, Biology Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; e-mail: tkkohno@ncc.go.jp.

0732-183X/10/2833-4945/$20.00

DOI: 10.1200/JCO.2010.30.5334

ABSTRACT

Purpose
To identify polymorphisms in DNA repair genes that affect responses to platinum-based doublet chemotherapy in patients with non–small-cell lung cancer (NSCLC).

Patients and Methods
In total, 640 patients with NSCLC who received platinum-based doublet chemotherapy in the National Cancer Center Hospital in Japan from 2000 to 2008 and whose responses were evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) participated in a study of the association between response and genotypes for 30 single nucleotide polymorphisms (SNPs) in 27 DNA repair genes. Candidate SNPs were selected in a discovery set of 201 patients, and their associations were validated in an independent set of 439 patients by prespecified *P* value criteria.

Results
Homozygotes for the minor allele TP53-72Pro of the Arg72Pro SNP in the *TP53* gene showed a better response rate (54.3%) than those for the major allele TP53-72Arg (29.1%; $P = 4.4 \times 10^{-5}$) irrespective of therapeutic regimens, and minor allele homozygotes had significantly longer progression-free and overall survivals than major allele homozygotes (hazard ratio [HR], 0.85; 95% CI, 0.74 to 0.98; $P = .020$; and HR, 0.86; 95% CI, 0.74 to 0.99; $P = .039$). Minor allele carriers for SNP Lys940Arg in the poly (ADP-ribose) polymerase 1 (*PARP1*) gene showed a better response rate to the paclitaxel regimen (45.8%) than to the gemcitabine regimen (10.5%; *P* for interaction = .019).

Conclusion
Polymorphisms in the *TP53* and *PARP1* genes are involved in inter-individual differences in the response to platinum-based doublet chemotherapy in patients with NSCLC.

J Clin Oncol 28:4945-4952. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Non–small-cell lung cancer (NSCLC) is a major cause of cancer-related death with 5-year survival rates of < 20%.[1] Cytotoxic chemotherapy is the standard care for patients with advanced NSCLC. The standards of therapeutic regimens are platinum-based doublets (platinum plus another agent).[2] The drugs paired with platinum include microtubule-targeted agents (paclitaxel, docetaxel, or vinorelbine) and DNA-damaging agents (gemcitabine or irinotecan). The efficacy of each combination has been demonstrated to be similar by a series of trials in unselected patients with response rates of 30% to 40%.[3-5] Therefore, predictive factors for the efficacy of these chemotherapy regimens are being investigated for the development of customized therapies.

Considering that agents that damage DNA or disturb chromosomal integrity are used for chemotherapy, activities that repair DNA or chromosome damage possibly influence the outcome of patients with NSCLC after chemotherapy. In fact, expression of *ERCC1*, which is involved in the repair of DNA adducts generated by platinum, has been shown to be a possible predictive factor for the efficacy of the postoperative cisplatin-based adjuvant chemotherapy in resected tumors.[6,7] More recently, a single nucleotide polymorphism (SNP) in the *ERCC1* gene, rs11615, which affects *ERCC1* mRNA levels, was suggested to be associated with response (ie, tumor regression) of patients with advanced NSCLC to platinum-based chemotherapy.[8] Since SNPs can be examined by using blood cells, they will be promising biomarkers in the clinical

decision-making process for patients with advanced NSCLC. Reports on the association of SNPs in several other DNA repair genes with prognosis of patients with NSCLC who received chemotherapy also suggested their associations with the outcome of the patients.[7,9-14] However, sample sizes were small (50 to 250 patients), and only four to 15 genomic polymorphisms were investigated in those studies. In addition, the data in each trial were not confirmed by an independent validation set. Therefore, clinical importance of these SNPs still remains unclear.

We previously searched for nonsynonymous (ie, associated with amino acid changes) SNPs in 36 DNA repair genes involved in diverse intracellular processes that maintain genome integrity and identified 29 SNPs in 26 DNA repair genes, whose minor allele frequencies were more than 5% in Japanese patients[15] (Table 1). Thus, in this study, we conducted a single-hospital–based retrospective analysis of 640 patients with NSCLC to elucidate associations of these 29 SNPs and the ERCC1 SNP above[8] with the patients' outcome after platinum-based doublet chemotherapy. To minimize type I errors, the significance of candidate SNPs picked up by the first discovery set were validated by using the second independent validation set. We chose the response evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST)[16] as the primary end point of outcome to search for predictive factors for the primary effect of chemotherapy.

Table 1. 30 SNPs in DNA Repair Genes

Pathway	Gene	SNP (rs number)	Amino Acid/ Nucleotide Change	Minor Allele Frequency				
				Japanese*	Japanese†	Chinese†	European†	African†
29 Nonsynonymous SNPs (associated with amino acid change)								
Base excision repair								
	PARP1	rs1805412	Val762Ala	0.40	0.46	0.48	0.17	0.01
		rs1136471	Lys940Arg	0.05	—	—	—	—
	APEX	rs1130409	Asp148Glu	0.38	0.32	0.46	0.51	0.28
	MBD4	rs140693	Glu346Lys	0.35	0.41	0.27	0.00	0.03
	MTH1	rs4866	Val83Met	0.09	—	—	—	—
	OGG1	rs1052133	Ser326Cys	0.48	0.52	0.50	0.22	0.14
	XRCC1	rs1799782	Arg194Trp	0.33	0.28	0.24	0.09	0.08
		rs25489	Arg280His	0.09	—	—	0.03	0.03
		rs25487	Arg399Gln	0.25	0.28	0.27	—	0.10
Nucleotide excision repair								
	XPG	rs17655	His1104Asp	0.42	0.48	0.56	0.73	0.46
	CSB	rs2228528	Gly399Asp	0.45	0.46	0.40	0.19	0.22
	XPC	rs2228001	Lys939Gln	0.40	0.34	0.38	0.41	0.26
	XPD	rs13181	Lys751Gln	0.05	0.08	0.06	0.33	0.18
Mismatch repair								
	MLH3	rs175080	Pro844Leu	0.18	0.14	0.13	0.43	0.41
	MSH3	rs26279	Thr1045Ala	0.24	0.22	0.37	0.22	0.40
	MSH6	rs1042821	Gly39Glu	0.32	—	—	—	—
DNA double-strand break repair								
	BRCA2	rs144848	Asn372His	0.22	0.31	0.21	0.29	0.13
	SNM1	rs3750898	His317Asp	0.26	0.26	0.10	0.27	0.74
	NBS1	rs1805794	Gln185Glu	0.50	0.46	0.49	0.28	0.16
	XRCC3	rs861539	Thr241Met	0.09	0.15	0.07	0.42	0.24
DNA damage response								
	TP53	rs1042522	Arg72Pro	0.33	0.23	0.49	0.41	0.67
DNA polymerase								
	POLD1	rs1726801	Arg119His	0.20	0.22	0.18	0.06	0.35
	POLI	rs8305	Thr731Ala	0.25	0.28	0.29	0.26	0.00
	REV1	rs3087386	Phe257Ser	0.33	0.30	0.37	0.50	0.30
	POLZ	rs462779	Thr1224Ile	0.35	0.43	0.49	0.82	0.38
Other pathways								
	BLM	rs28384991	Thr298Met	0.09	—	—	—	—
	FANCA	rs2239359	Ser501Gly	0.17	0.16	0.21	0.62	0.33
	FANCG	rs2237857	Thr297Ile	0.12	0.13	0.01	0.00	0.14
	WRN	rs1346044	Cys1367Arg	0.09	0.07	0.08	0.23	0.15
One synonymous SNP (not associated with amino acid change)								
Nucleotide excision repair								
	ERCC1	rs11615	C118T	—	0.29‡	0.22	0.65	0.02

Abbreviation: SNP, single nucleotide polymorphism.
*Frequency in Japanese determined by Sakiyama et al.[15]
†Frequency determined by the HapMap project.
‡Frequency in Japanese (T. Kohno, unpublished data).

PATIENTS AND METHODS

Selection of Study Population and Acquisition of Clinical Information

In total, 987 patients with NSCLC with clinical stages IIIA, IIIB, and IV tumors, who had not received prior platinum-based chemotherapy, were given platinum-based chemotherapy at the National Cancer Center Hospital in Tokyo, Japan, from 2000 to 2008 (Fig 1A). Clinical information was obtained by attending physicians and nurses. Of the 987 patients, 640 were eligible for the study according to the following criteria: they were not indicated for definitive chemoradiotherapy; they received a platinum-based doublet but not single or triplet chemotherapy; and their tumor response was evaluable according to RECIST[16] on the basis of data from computed tomography scans. However, those with clinical or radiologic evidence of early progression, such as emergence of new lesions, were included as patients with progressive disease (PD) in the analysis, even when unaccompanied by corresponding computed tomography scans, according to the definition in RECIST.[16] All patients were Japanese and were diagnosed with adenocarcinoma (ADC), squamous cell carcinoma (SQC), or other histologic types of NSCLC according to WHO classification[17,18] (Table 2).

Written informed consent was obtained from all patients for the use of blood cells for the analysis of genetic polymorphisms in association with clinical findings, including response to chemotherapy. Thus, 201 patients in the discovery set received therapy from 2000 to 2004, and 439 patients in the validation set received therapy from 2004 to 2008. Information on response in a subset of patients was obtained from the data in clinical trials conducted at the National Cancer Center Hospital.[3,19,20] This study was approved by the institutional review boards of the National Cancer Center. Smoking habit was recorded by pack-years. Patients with pack-years > 0 were defined as smokers, including both former and current smokers. Patients who report no smoking history (ie, pack-years = 0) were defined as never-smokers.

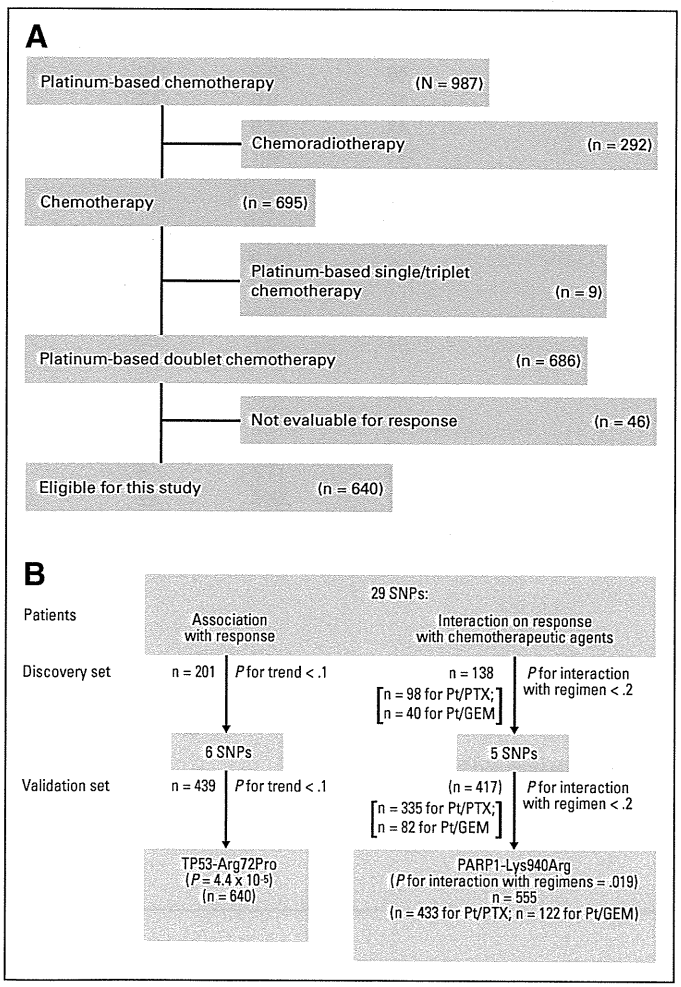

Fig 1. Patients and strategy. (A) Selection of eligible cases. (B) A two-phase screening of single nucleotide polymorphisms (SNPs) associated with responses to platinum-based doublet chemotherapy. Pt, platinum; PTX, paclitaxel; GEM, gemcitabine.

Chemotherapy

Patients were treated with one of the following regimens: (1) paclitaxel 200 mg/m^2 followed by cisplatin 80 mg/m^2, carboplatin at a dose calculated to produce an area under the serum concentration-time curve of 6.0 min · mg/mL, or nedaplatin 100 mg/m^2 on day 1, repeated every 3 weeks; (2) docetaxel 60 mg/m^2 followed by cisplatin 80 mg/m^2 on day 1, repeated every 3 weeks; (3) vinorelbine 25 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 on day 1, repeated every 3 weeks; (4) gemcitabine 1,000 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 or carboplatin to area under the serum concentration-time curve of 5.0 min · mg/mL on day 1, repeated every 3 weeks; or (5) irinotecan 60 mg/m^2 on days 1, 8, and 15 and cisplatin 80 mg/m^2 on day 1, repeated every 4 weeks. Each treatment was repeated for two or more cycles unless the patient met the criteria for PD or experienced unacceptable toxicity. Chemotherapy dosage was modified by toxicities in subsequent courses.

Genetic Analysis

A 20 mL whole-blood sample was obtained from each patient, and genomic DNA was extracted from whole-blood cells.[15] Genotyping for 30 SNPs in 27 genes was performed by pyrosequencing or TaqMan methods as previously described.[15,21]

Statistical Analysis

Patients were divided into two categories: responders were those with complete response and partial response, and nonresponders were those with stable disease and PD. Odds ratios (ORs) and 95% CIs for the response (ie, responder *v* nonresponder) according to genotypes were calculated as a measure of difference in the response rate against therapy. ORs were calculated by adjusting sex (male *v* female), age (increase by 10 years), performance status (0 *v* 1 to 2), smoking status (never-smoker *v* smoker), stage (III *v* IV), and chemotherapy (platinum plus a DNA-damaging agent *v* platinum plus a microtubule-targeting agent) by using an unconditional logistic regression analysis.[22] *P* value by the trend test was also calculated by using an unconditional logistic regression analysis under the same adjustments as above. Differences in the response between two chemotherapeutic regimens according to genotypes were examined by calculating *P* values for interaction with the regimens on the trend of OR for response.

A two-phase screening was used to search for SNPs associated with the response to chemotherapy (Fig 1B). In the first phase, 29 SNPs were examined for associations with the response and differences in the association according to regimens in 201 and 138 patients (for whom paclitaxel or gemcitabine therapy was used, respectively) in the discovery set. In the second phase, SNPs that showed *P* values < .1 by the trend test for association with the response and *P* values < .2 for interaction with the regimen were subjected to genotyping of 439 and 417 patients (for whom paclitaxel or gemcitabine was used, respectively) in the validation set. SNPs that showed *P* values < .1 for association with the response and *P* values < .2 for interaction with the regimen in patients in the validation set were further subjected to analysis in all 640 and 555 patients, respectively. Progression-free survival (PFS) was defined as the period from the first day of chemotherapy to the date of documentation of disease progression by RECIST and overall survival (OS) was defined as the period from the first day of chemotherapy to death. Hazard ratios (HRs) for PFS and OS and 95% CIs were calculated by using multivariate Cox proportional hazards models with adjustment for sex, age, histology, performance status, smoking status, clinical stage, and treatment as above. Statistical analyses were performed using JMP version 8.0 software (SAS Institute, Cary, NC). A level of $P < .05$ was considered significant, whereas a level of $P < .10$ was considered marginal.

Table 2. Patient Characteristics

Variant	All			Discovery Set*		Validation Set	
	No.	%	95% CI	No.	%	No.	%
Total patients	640			201		439	
Age, years							
Mean	57.9			57.2		58.2	
Range	22-78			22-78		26-74	
± Standard deviation	9.2			10.0		9.1	
Sex							
Male	402	62.8		136	67.7	266	60.6
Female	238	37.2		65	32.3	173	39.4
ECOG performance status							
0	218	34.1		46	22.9	172	39.2
1	402	62.8		153	76.1	249	56.7
2	20	3.1		2	1.0	18	4.1
Histologic cell type							
Adenocarcinoma	549	85.8		167	83.1	382	87.0
Squamous cell carcinoma	84	13.1		34	16.9	50	11.4
Others	7	1.1		0	0.0	7	1.6
Smoking habit							
Never-smoker	233	36.4		74	36.8	159	36.2
Smoker	407	63.6		127	63.2	280	63.8
Pack-years of smokers							
Mean	46.3			45.9		46.5	
± Standard deviation	29.6			29.4		29.7	
Stage							
III	172	26.9		60	29.9	112	25.5
IIIA	24	3.8		12	6.0	12	2.7
IIIB	148	23.1		48	23.9	100	22.8
IV	468	73.1		141	70.1	327	74.5
Tumor response							
Responder	231	36.1		74	36.8	157	35.8
CR	4	0.6		0	0.0	4	0.9
PR	227	35.5		74	36.8	153	34.9
Non-responder	409	63.9		127	63.2	282	64.2
SD	232	36.3		70	34.8	162	36.9
PD	177	27.7		57	28.4	120	27.3
Platinum-based regimens							
Platinum + a microtubule-targeted agent	476	74.4		129	64.2	347	79.0
Paclitaxel†	433	67.7		98	48.8	335	76.3
Docetaxel‡	8	1.3		2	1.0	6	1.4
Vinorelbine§	35	5.5		29	14.4	6	1.4
Platinum + a DNA-damaging agent	164	25.6		72	35.8	92	21.0
Gemcitabine¶	122	19.1		40	19.9	82	18.7
Irinotecan‖	42	6.6		32	15.9	10	2.3
PFS, median month							
Platinum + Paclitaxel	4.7		4.2 to 5.3				
Platinum + Gemcitabine	4.6		3.8 to 5.4				
Responder	6.1		5.7 to 6.4				
Nonresponder	3.0		2.7 to 3.3				

Abbreviations: ECOG, Eastern Cooperative Oncology Group; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival.

*Genotype for 29 nonsynonymous DNA repair gene single nucleotide polymorphisms were determined by Sakiyama et al.[15]

†Cisplatin or carboplatin or nedaplatin + paclitaxel.

‡Cisplatin + docetaxel.

§Cisplatin + vinorelbine.

¶Cisplatin or carboplatin + gemcitabine.

‖Cisplatin + irinotecan.

RESULTS

Association of a TP53-Arg72Pro SNP With Response to Platinum-Based Doublet Chemotherapy

Among 987 patients with NSCLC who were treated with platinum-based chemotherapy, 640 were eligible for this study (Fig 1A). Characteristics of these patients are summarized in Table 2. Genotypes for the 29 nonsynonymous SNPs in 26 DNA repair genes had been determined in 201 of the 640 patients in our previous study[15] (the discovery set in Table 2). Therefore, associations of these 29 SNPs with responses to chemotherapy were first investigated in these patients (Fig 1B). Six of the 29 SNPs fulfilled the criteria described above ($P < .1$ by the trend test; Appendix Table A1, online only); thus, they were further genotyped in the remaining 439 patients (the validation set in Table 2). Only one SNP, TP53-Arg72Pro, reproducibly showed an association that met the criteria ($P < .1$; Fig 1B and Appendix Table A1). In the analysis of all 640 patients, TP53-72Pro, the minor allele, was associated with a better response ($P = 9.5 \times 10^{-5}$ by the trend test; Table 3), and response rates increased according to the increase in the number of minor alleles (Fig 2A). Minor allele homozygotes showed a better response rate (54.3%) than major allele homozygotes (29.1%; $P = 4.4 \times 10^{-5}$). The association remained significant after Bonferroni correction (ie, $< 0.05/29 = 1.7 \times 10^{-3}$). Response rates of heterozygotes and homozygotes for the TP53-72Pro allele were higher in SQC than in ADC (Fig 2A and Table 3).

In the Cox proportional hazard model, minor allele homozygotes showed a significantly longer PFS than major allele homozygotes (HR, 0.85; 95% CI, 0.74 to 0.98; $P = .020$). The HR for progression of these homozygotes in SQC (HR, 0.67; 95% CI, 0.45 to 0.98; $P = .041$) was lower than that in ADC (HR, 0.89; 95% CI, 0.76 to 1.03; $P = .13$). Minor allele homozygotes showed a significantly longer OS than major allele homozygotes (HR, 0.86; 95% CI, 0.74 to 0.99; $P = .039$). The HR for death of these homozygotes in SQC (HR, 0.66; 95% CI, 0.43 to 0.98; $P = .037$) was lower than that in ADC (HR, 0.87; 95% CI, 0.74 to 1.02; $P = .13$).

SNP rs11615 (C118T) in the *ERCC1* gene was reported to be associated with response to platinum-based chemotherapy of NSCLC[8]; thus, it was also examined for association with response in all 640 patients. Minor allele homozygotes for the *ERCC1* SNP showed a higher response rate than others, consistent with a recent report[8]; however, the association was not statistically significant (Appendix Table A2, online only).

Differential Response According to Chemotherapeutic Regimens by PARP1 Genotypes

We next investigated whether or not SNPs in DNA repair genes affect responses differentially according to chemotherapeutic agents. Paclitaxel (433 patients; 68%) and gemcitabine (122 patients; 19%) were the most and second-most commonly used drugs in the platinum-based regimens (other drugs were also used but less frequently [< 10%; Table 2]). Therefore, differences in the response among the

Table 3. Association of *TP53* Genotypes With Response to Chemotherapy in 640 Patients With NSCLC

NSCLC	Genotype	Nonresponders No.	Nonresponders %	Responders No.	Responders %	Response Rate (%)*	OR	95% CI	P	P by Trend Test
All	Arg/Arg	175	42.8	72	31.2	29.1	Reference			9.5×10^{-5}
	Arg/Pro	197	48.2	115	49.8	36.9	1.38	0.96 to 1.99	.082†	
	Pro/Pro	37	9.0	44	19.0	54.3	3.02	1.77 to 5.18	4.4×10^{-5}†	
	Dominant						1.63	1.15 to 2.30	.0053†	
	Recessive						2.48	1.54 to 4.04	2.1×10^{-4}†	
Adenocarcinoma	Arg/Arg	152	42.2	64	33.9	29.6	Reference			.0024
	Arg/Pro	176	48.9	90	47.6	33.8	1.19	0.81 to 1.77	.38‡	
	Pro/Pro	32	8.9	35	18.5	52.2	2.67	1.50 to 4.81	8.7×10^{-4}‡	
	Dominant						1.42	0.98 to 2.07	.062‡	
	Recessive						2.44	1.44 to 4.15	9.2×10^{-4}‡	
Squamous cell carcinoma	Arg/Arg	21	46.7	7	17.9	25.0	Reference			.0032
	Arg/Pro	19	42.2	23	59.0	54.8	3.63	1.10 to 13.5	.033‡	
	Pro/Pro	5	11.1	9	23.1	64.3	8.71	1.64 to 62.5	.010‡	
	Dominant						4.62	1.52 to 16.3	.0062‡	
	Recessive						3.85	1.02 to 17.6	.047‡	
Smoker	Arg/Arg	98	39.5	44	27.7	31.0	Reference			.0084
	Arg/Pro	124	50.0	88	55.3	41.5	1.52	0.97 to 2.41	.069§	
	Pro/Pro	26	10.5	27	17.0	50.9	2.31	1.19 to 4.50	.013§	
	Dominant						1.65	1.07 to 2.57	.023§	
	Recessive						1.78	0.99 to 3.23	.056§	
Never-smoker	Arg/Arg	77	47.0	28	38.9	26.7	Reference			.0052
	Arg/Pro	73	44.5	27	37.5	27.0	1.06	0.55 to 2.02	.87§	
	Pro/Pro	11	6.7	17	23.6	60.7	5.31	2.00 to 15.3	6.8×10^{-4}§	
	Dominant	161					1.56	0.86 to 2.86	.14§	
	Recessive						4.76	2.02 to 11.8	3.6×10^{-4}§	

Abbreviations: NSCLC, non–small-cell lung cancer; OR, odds ratio.
*Fraction of responder.
†OR for responder against nonresponder adjusted for sex, age, histology, smoking status, clinical stage, performance status, and treatment.
‡OR for responder against nonresponder adjusted for sex, age, smoking status, clinical stage, performance status, and treatment.
§OR for responder against nonresponder adjusted for sex, age, histology, clinical stage, performance status, and treatment.

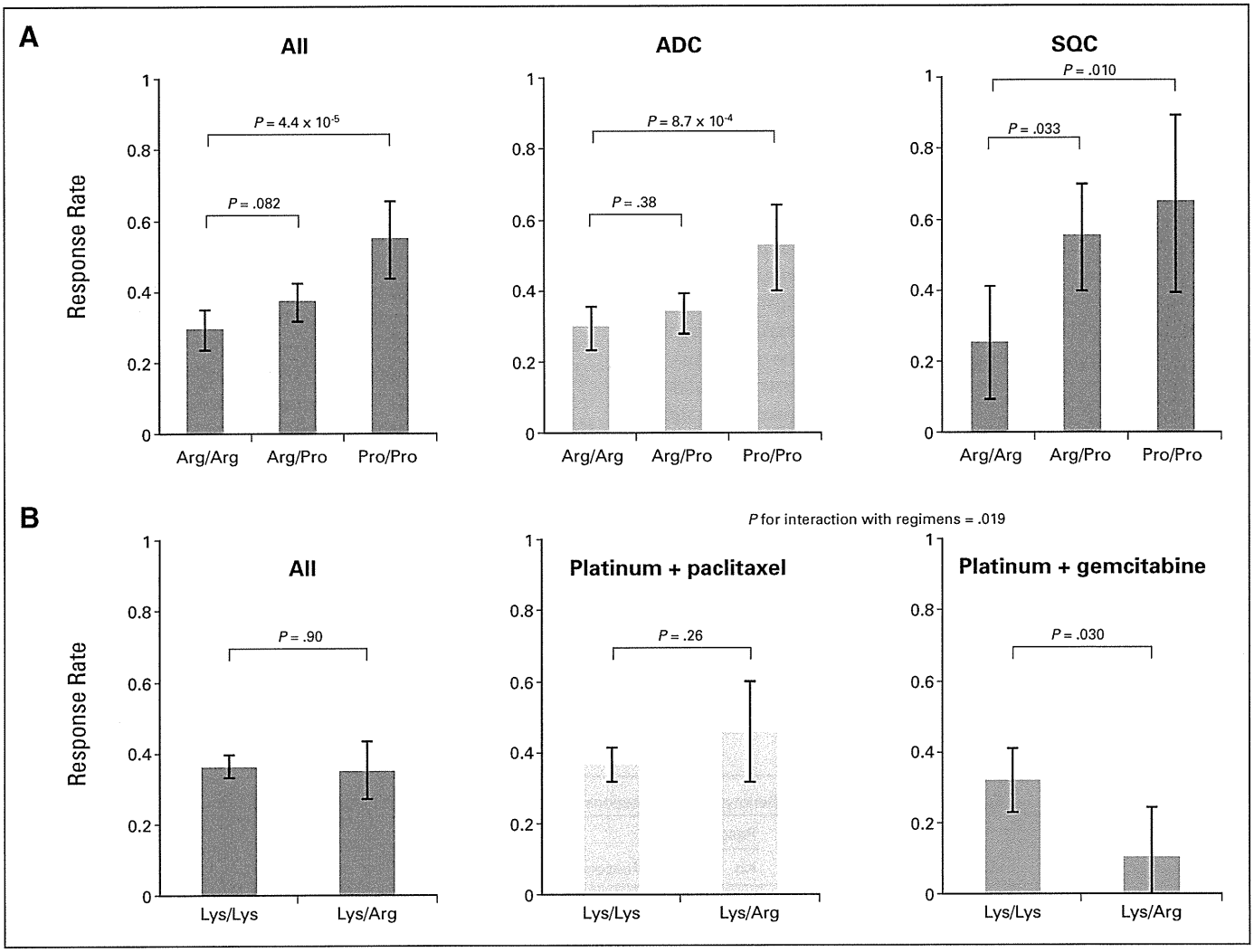

Fig 2. (A) Response rates according to *TP53* genotypes in (left) all patients and those with (middle) aenocarcinoma (ADC) and (right) squamous cell carcinoma (SQC). (B) Response rates according to *PARP1* genotypes in (left) all patients and those treated with (middle) platinum plus pacliteaxel or (right) platinum plus gemcitabine. Response rate is shown with its sampling variations estimated by 95% CI.

agents according to genotypes were investigated in 555 patients who received chemotherapy with either of these two regimens.

Among 201 patients in the discovery set, 138 received chemotherapy with regimens using paclitaxel (98 patients) or gemcitabine (40 patients; Fig 1B). Five of the 29 SNPs met the criteria in these 138 patients ($P < .2$ for interaction). Therefore, these five SNPs were further genotyped for 417 patients who received chemotherapy with regimens using paclitaxel (335 patients) or gemcitabine (82 patients) among 439 patients in the validation set. Only one SNP, poly (ADP-ribose) polymerase 1 (PARP1) -Lys940Arg, reproducibly showed $P < .2$ for interaction (Appendix Table A3, online only). This SNP showed a statistically significant interaction with the regimens on the response when analyzed in all 555 patients ($P = .019$ for interaction; Fig 1B, Appendix Table A4, online only), although the association did not remain significant after Bonferroni correction (ie, > 0.05 of 29 SNPs tested $= 1.7 \times 10^{-3}$). Heterozygotes for this SNP showed a better response rate to the paclitaxel regimen (45.8%) than to the gemcitabine regimen (10.5%; Fig 2B). There were no minor allele homozygotes for this SNP in this population.

PFS according to the PARP1-Lys940Arg genotype was compared between the two regimens. In the Cox proportional hazard model, the risk for progression of major allele homozygotes with the platinum/paclitaxel treatment was similar to that with the platinum/gemcitabin treatment (HR, 0.97; 95% CI, 0.86 to 1.09; $P = .60$). Conversely, the risk of heterozygotes with the platinum/paclitaxel treatment was smaller than that with the platinum/gemcitabine treatment, although it was not statistically significant (HR, 0.82; 95% CI, 0.59 to 1.17; $P = .27$). SNPs in *TP53* and *ERCC1* did not show differential associations according to regimens (Appendix Table A4).

DISCUSSION

An SNP in the *TP53* genes was shown to be associated with the response to platinum-based doublet chemotherapy. In this study, association results obtained by the discovery set were confirmed by using an independent validation set. The association of the p53-72Pro allele with a better response to platinum-based doublet chemotherapy

retained statistical significance after Bonferroni correction. Therefore, the results strongly indicate the importance of p53-Arg72Pro SNP as a determinant for the response to platinum-based chemotherapy.

TP53 is a tumor suppressor gene somatically mutated in 40% to 70% of NSCLCs.[23] p53-72Arg protein has a greater activity to induce apoptosis than p53-72Pro protein[24]; however, the relationship was reported as being the reverse in mutant p53 proteins.[25,26] p73, a p53-related protein, plays a role in apoptosis in anticancer agents for cancer cells carrying *TP53* mutations; however, its function is abrogated by mutant p53 proteins. The abrogating activity is greater in mutant p53 proteins with the Arg residue at codon 72 than in those with the Pro residue.[25,26] In an analysis of 25 patients with head and neck cancer, those with a *TP53* mutation on the 72Pro allele showed a better response than those with a mutation on the 72Arg allele with cisplatin-based chemoradiotherapy.[25] Similarly, in this study, the TP53-72Pro allele appeared to confer a better response to platinum-based doublet chemotherapy in patients with NSCLC (Fig 2A). In a previous study,[11] patients with NSCLC who carry the TP53-72Pro allele also showed a better OS after cisplatin-gemcitabine treatment, although the association did not reach statistical significance. These results indicate that p53 mutants with the Pro residue at codon 72 only weakly inhibit the function of p73 protein in NSCLC cells and therefore efficiently induce apoptosis of NSCLC cells treated with platinum and other anticancer agents. In fact, the effect of this SNP was more apparent in patients with SQC than in patients with ADC (Fig 1A), consistent with the fact that *TP53* mutations are more frequent in SQC than in ADC.[27] Since tumor specimens for examination of somatic *TP53* mutations were not available for these patients, *TP53* status in their tumor cells could not be determined. Therefore, we could not conclude whether this differential association was really due to differences in *TP53* mutations. An association study of patients with NSCLC informative for somatic *TP53* mutation will provide a more complete picture of the role of *TP53* SNP in chemotherapeutic responses.

The PARP1-Lys940Arg genotype was suggested to differentially affect the response according to chemotherapeutic agents (Fig 2B), although the association was not significant after Bonferroni correction and needs validation. The *PARP1* gene encodes poly (ADP-ribose) polymerase 1, which regulates multiple processes for DNA repair, such as DNA strand break repair.[28] It is noted that suppression of PARP activity has been recognized as a method of tumor suppression in breast and other cancers[29] and that a PARP inhibitor enhanced the cytotoxic activity of gemcitabine.[30] The biologic significance of the PARP1-Lys940Arg SNP is unknown at present; however, the lysine-arginine residue at codon 940 is located in the catalytic domain of the PARP1 protein.[31] Therefore, this polymorphism may cause differences in the activity of PARP1 protein that affect the response to some chemotherapeutic agents, in particular to DNA-damaging agents.

Interestingly, the frequencies of the TP53-72Pro allele are known to be different among ethnic populations, although those of the PARP1-940Arg allele in other ethnic populations are unknown at present (Table 1). Therefore, examination of these two SNPs in NSCLC patient populations other than Japanese will also help elucidate the mechanism of interethnic differences in the outcome of patients after chemotherapy, as recently discussed.[32]

Identification of polymorphisms associated with drug toxicities is also important to develop customized chemotherapies. For instance, the *UGT1A1* gene polymorphisms are known to be associated with the toxicity of irinotecan, such as neutropenia.[33] In this study, the *TP53* and *PARP1* SNPs were not associated with grade 4 hematologic toxicities, including neutropenia (data not shown). Therefore, genetic factors responsible for response are likely to be different from those for toxicity. In addition, associations of these two SNPs with responses were not significantly different according to smoking habit ($P > .05$ for interaction with smoking; for *TP53*, see Table 3); therefore, these SNPs are likely to contribute to the response irrespective of smoking.

Our study has several limitations. This is a single-institution retrospective study with various therapeutic regimens. Therefore, the effects of SNPs on differential responses according to chemotherapeutic agents were only preliminarily investigated. The results should be confirmed by a larger, preferably prospective, cohort using a defined set of agents. More extensive analyses of interaction between SNPs and responses to chemotherapeutic agents will also be worth performing. Another limitation of this study is that, although the *TP53* polymorphism was significantly associated with response to chemotherapy, differences in PFS and OS were only modest. We chose the response as the primary end point of efficacy to pick up subgroups for which chemotherapy does work. Although this information would be potentially valuable, clinical response alone would be inadequate to improve the outcome of patients with advanced NSCLC. Therefore, investigation of polymorphisms in other genes might provide more information for individually optimized chemotherapy. Indeed, a few other SNPs in DNA repair genes have been reported to be associated with prognosis of patients with NSCLC.[7,9-14] In addition to ERCC1-118T, the APEX-148Asp, XRCC1-399Arg, and XPD-751Gln alleles, which had been reported to be associated with favorable prognosis of patients,[9,13,14] were consistently more frequent in responders than in nonresponders in our study population (Appendix Table A2), although these SNPs did not fulfill the criteria as validated predictive factors in this study.

In conclusion, our extensive analysis of 30 SNPs in 27 DNA repair genes identified the *TP53* and *PARP1* SNPs as strong candidates for defining inter-individual differences in the response to platinum-based chemotherapy of NSCLC. Our results indicate the significance of SNPs in DNA repair genes in the outcome of patients with NSCLC and also imply the utility of these SNPs as predictive markers for responses to chemotherapy. Further investigation is warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Takashi Kohno, Jun Yokota, Hideo Kunitoh
Financial support: Takashi Kohno, Tomohide Tamura, Jun Yokota
Provision of study materials or patients: Chiharu Tanai, Yasushi Goto, Hiroshi Nokihara, Noboru Yamamoto, Ikuo Sekine, Yuichiro Ohe, Tomohide Tamura, Hideo Kunitoh
Collection and assembly of data: Kouya Shiraishi, Chiharu Tanai, Yasushi Goto, Koji Tsuta
Data analysis and interpretation: Kouya Shiraishi, Takashi Kohno, Aya Kuchiba, Seiichiro Yamamoto, Jun Yokota, Hideo Kunitoh
Manuscript writing: Kouya Shiraishi, Takashi Kohno, Jun Yokota, Hideo Kunitoh

Final approval of manuscript: Kouya Shiraishi, Takashi Kohno, Chiharu Tanai, Yasushi Goto, Aya Kuchiba, Seiichiro Yamamoto, Koji Tsuta, Hiroshi Nokihara, Noboru Yamamoto, Ikuo Sekine, Yuichiro Ohe, Tomohide Tamura, Jun Yokota, Hideo Kunitoh

REFERENCES

1. Parkin DM, Bray F, Ferlay J, et al: Global cancer statistics, 2002. CA Cancer J Clin 55:74-108, 2005
2. Goffin J, Lacchetti C, Ellis PM, et al: First-line systemic chemotherapy in the treatment of advanced non-small cell lung cancer: A systematic review. J Thorac Oncol 5:260-274, 2010
3. Ohe Y, Ohashi Y, Kubota K, et al: Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Ann Oncol 18:317-323, 2007
4. Scagliotti GV, De Marinis F, Rinaldi M, et al: Phase III randomized trial comparing three platinum-based doublets in advanced non-small-cell lung cancer. J Clin Oncol 20:4285-4291, 2002
5. Schiller JH, Harrington D, Belani CP, et al: Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 346:92-98, 2002
6. Olaussen KA, Dunant A, Fouret P, et al: DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 355:983-991, 2006
7. Camps C, Sirera R, Iranzo V, et al: Gene expression and polymorphisms of DNA repair enzymes: Cancer susceptibility and response to chemotherapy. Clin Lung Cancer 8:369-375, 2007
8. Wei SZ, Zhan P, Shi MQ, et al: Predictive value of ERCC1 and XPD polymorphism in patients with advanced non-small cell lung cancer receiving platinum-based chemotherapy: A systematic review and meta-analysis. Med Oncol [epub ahead of print on February 9, 2010]
9. Gurubhagavatula S, Liu G, Park S, et al: XPD and XRCC1 genetic polymorphisms are prognostic factors in advanced non-small-cell lung cancer patients treated with platinum chemotherapy. J Clin Oncol 22:2594-2601, 2004
10. Isla D, Sarries C, Rosell R, et al: Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. Ann Oncol 15:1194-1203, 2004
11. de las Peñas R, Sanchez-Ronco M, Alberola V, et al: Polymorphisms in DNA repair genes modulate survival in cisplatin/gemcitabine-treated non-small-cell lung cancer patients. Ann Oncol 17:668-675, 2006
12. Giachino DF, Ghio P, Regazzoni S, et al: Prospective assessment of XPD Lys751Gln and XRCC1 Arg399Gln single nucleotide polymorphisms in lung cancer. Clin Cancer Res 13:2876-2881, 2007
13. Matakidou A, el Galta R, Webb EL, et al: Genetic variation in the DNA repair genes is predictive of outcome in lung cancer. Hum Mol Genet 16:2333-2340, 2007
14. Wu X, Lu C, Ye Y, et al: Germline genetic variations in drug action pathways predict clinical outcomes in advanced lung cancer treated with platinum-based chemotherapy. Pharmacogenet Genomics 18:955-965, 2008
15. Sakiyama T, Kohno T, Mimaki S, et al: Association of amino acid substitution polymorphisms in DNA repair genes TP53, POLI, REV1 and LIG4 with lung cancer risk. Int J Cancer 114:730-737, 2005
16. Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216, 2000
17. Travis W, Colby, TV, Corrin, B, et al: Histological Typing of Lung and Pleural Tumors (ed 3). Heidelberg, Germany, Springer-Verlag, 1999
18. Brambilla E, Travis WD, Colby TV, et al: The new World Health Organization classification of lung tumours. Eur Respir J 18:1059-1068, 2001
19. Kawaishi M, Fujiwara Y, Fukui T, et al: Circulating endothelial cells in non-small cell lung cancer patients treated with carboplatin and paclitaxel. J Thorac Oncol 4:208-213, 2009
20. Sekine I, Nokihara H, Horiike A, et al: Phase I study of cisplatin analogue nedaplatin (254-S) and paclitaxel in patients with unresectable squamous cell carcinoma. Br J Cancer 90:1125-1128, 2004
21. Shiraishi K, Kohno T, Kunitoh H, et al: Contribution of nicotine acetylcholine receptor polymorphisms to lung cancer risk in a smoking-independent manner in the Japanese. Carcinogenesis 30:65-70, 2009
22. Breslow NE, Day NE: Statistical methods in cancer research: Volume I—The analysis of case-control studies. IARC Scientific Publication No. 32, 1980, pp 5-338
23. Weston A, Perrin LS, Forrester K, et al: Allelic frequency of a p53 polymorphism in human lung cancer. Cancer Epidemiol Biomarkers Prev 1:481-483, 1992
24. Dumont P, Leu JI, Della Pietra AC 3rd, et al: The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 33:357-365, 2003
25. Bergamaschi D, Gasco M, Hiller L, et al: p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 3:387-402, 2003
26. Vikhanskaya F, Siddique MM, Kei Lee M, et al: Evaluation of the combined effect of p53 codon 72 polymorphism and hotspot mutations in response to anticancer drugs. Clin Cancer Res 11:4348-4356, 2005
27. The International Agency for Research on Cancer: IARC TP53 Database. http://www-p53.iarc.fr/
28. Amé JC, Spenlehauer C, de Murcia G: The PARP superfamily. Bioessays 26:882-893, 2004
29. Iglehart JD, Silver DP: Synthetic lethality: A new direction in cancer-drug development. N Engl J Med 361:189-191, 2009
30. Jacob DA, Bahra M, Langrehr JM, et al: Combination therapy of poly (ADP-ribose) polymerase inhibitor 3-aminobenzamide and gemcitabine shows strong antitumor activity in pancreatic cancer cells. J Gastroenterol Hepatol 22:738-748, 2007
31. Cao WH, Wang X, Frappart L, et al: Analysis of genetic variants of the poly(ADP-ribose) polymerase-1 gene in breast cancer in French patients. Mutat Res 632:20-28, 2007
32. Gandara DR, Kawaguchi T, Crowley J, et al: Japanese-US common-arm analysis of paclitaxel plus carboplatin in advanced non-small-cell lung cancer: A model for assessing population-related pharmacogenomics. J Clin Oncol 27:3540-3546, 2009
33. Bosch TM: Pharmacogenomics of drug-metabolizing enzymes and drug transporters in chemotherapy. Methods Mol Biol 448:63-76, 2008

Cancer Science
The official journal of the Japanese Cancer Association JCA

Review Article

Epithelial–mesenchymal transition in cancer development and its clinical significance

Masaaki Iwatsuki,[1,2] Koshi Mimori,[1] Takehiko Yokobori,[1] Hideshi Ishi,[1,3] Toru Beppu,[2] Shoji Nakamori,[4] Hideo Baba[2] and Masaki Mori[1,3,5]

[1]Department of Surgical Oncology, Medical Institute of Bioregulation, Kyushu University, Beppu; [2]Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto; [3]Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka; [4]Department of Surgery, Osaka National Hospital, Osaka, Japan

(Received September 07, 2009/Revised October 21, 2009/Accepted October 21, 2009/Online publication November 24, 2009)

The epithelial–mesenchymal transition (EMT) plays a critical role in embryonic development. EMT is also involved in cancer progression and metastasis and it is probable that a common molecular mechanism is shared by these processes. Cancer cells undergoing EMT can acquire invasive properties and enter the surrounding stroma, resulting in the creation of a favorable microenvironment for cancer progression and metastasis. Furthermore, the acquisition of EMT features has been associated with chemoresistance which could give rise to recurrence and metastasis after standard chemotherapeutic treatment. Thus, EMT could be closely involved in carcinogenesis, invasion, metastasis, recurrence, and chemoresistance. Research into EMT and its role in cancer pathogenesis has progressed rapidly and it is now hypothesized that novel concepts such as cancer stem cells and microRNA could be involved in EMT. However, the involvement of EMT varies greatly among cancer types, and much remains to be learned. In this review, we present recent findings regarding the involvement of EMT in cancer progression and metastasis and provide a perspective from clinical and translational viewpoints. (*Cancer Sci* 2010; 101: 293–299)

Development of distant metastases is the final stage of solid cancer progression and is responsible for the majority of cancer-related deaths.[1] Distant metastasis alone or with concurrent locoregional recurrence accounts for nearly 80% of all first relapses in women with breast cancer.[2] While clinically of great importance, the biology of metastasis remains unsolved. The process of tumor metastasis consists of multiple steps, all of which are required to achieve tumor spreading.[3,4] First, cancer cells escape from the primary tumor site. Next, cancer cells invade the tumor stroma and enter the blood circulation directly or the lymphatic system via intravasation. Most circulating cancer cells undergo apoptosis due to anoikis conditions.[5] If cancer cells survive in circulation they may reach more suitable sites by attaching to endothelial cells and extravasating from the circulation into the surrounding tissues. Finally, distal colonization requires that cancer cells invade and grow in the new environment.

Recently, the concept of the epithelial–mesenchymal transition (EMT), as developed in the field of embryology, has been extended to cancer progression and metastasis.[6,7] *In vitro* and experimental animal model data now support the role of EMT in metastasis, concepts supported by analyses of clinical samples. Indeed, the biology of EMT has been clarified in tumor samples through use of EMT-associated markers, such as mesenchymal-specific markers (i.e. vimentin and fibronectin),[8,9] epithelial specific markers (i.e. E-cadherin and cytokeratin),[10,11] and transcription factors (i.e. SNAIL and SLUG).[12]

Most recently, several intriguing studies have described the novel mechanism underlying EMT activation. In the current study, we will discuss the role of small non-coding RNA (microRNA) in regulating EMT-related genes.[13–15] Furthermore, Mani *et al.* disclosed that EMT could generate breast cancer cells with stem cell-like characteristics.[16] Here, we update and discuss recent progress in studies of EMT. These new data improve our understanding of the mechanisms of cancer progression and metastasis as well as therapy resistance. This new information may lead to development of novel clinical targets and improve the clinical management of cancer patients.

Involvement of EMT in Cancer Progression

In the 1980s, Greenburg and Hey first analyzed EMT-associated changes in cell phenotype and mesenchymal states in adult and embryonic epithelia.[17] EMT and the inverse process of mesenchymal–epithelial transition (MET) are major embryological mechanisms for tissue remodeling, as in gastrulation and segment formation.[18] The process of EMT consists of multiple steps.[19,20] First, cell–cell adhesion disintegrates with the loss of epithelial markers such as E-cadherin and the gain of mesenchymal markers such as vimentin. Next, there is a loss of basoapical polarization and the acquisition of front-rear polarization. Then, the cytoskeleton undergoes remodeling, with changes in cortical actin and actin stress fibers. Finally, cell-matrix adhesion is altered, with activation of proteolytic enzymes such as matrix metalloproteases. Note that the process of metastasis in epithelial cancer also consists of multiple steps.[3,4] That is, cells detach from the primary tumor and invade the surrounding tumor stroma. They subsequently enter into the circulation and reach new metastatic sites. Therefore, the process of EMT during cancer progression and metastasis closely resembles that observed in embryologic development. Accordingly, molecular analyses based on EMT in embryology have been applied to cancer progression.

In the 1990s, accumulating evidence indicated that EMT was associated with cancer progression.[7] Indeed, these transformations may be associated with EMT-related signal pathways during development.[7,21] However, Boyer *et al.* stated that EMT during development depends on additional activities of distinct and specific signaling molecules which are highly controlled spatially and temporally, and which do not occur under normal circumstances. On the other hand, EMT in cancer progression could be due to autonomous oncogenic activation of signaling molecules without additional stimulation.[22] Therefore,

[5]To whom correspondence should be addressed.
E-mail: mmori@gesurg.med.osaka-u.ac.jp

doi: 10.1111/j.1349-7006.2009.01419.x

comparisons of EMT signaling pathways in embryological development and cancer progression may make it possible to identify novel pathways specific to cancer progression and to suggest new therapeutic strategies in cancer therapy.[23]

The Molecular Mechanism of EMT in Cancer Progression

Multiple complex signaling systems are required for induction of EMT because epithelial cells undergoing EMT must undergo both functional and morphologic changes. Studies of the crosstalk among the intracellular signal networks could help us to understand the mechanisms regulating EMT. Here, we discuss the regulation of representative molecules, E-cadherin, a major EMT inducer, transforming growth factor-β (TGF-β) signal pathways, and microRNA regulation reported in recent studies (Fig. 1).

E-cadherin regulation. One of the characteristic findings in EMT is the loss of cell–cell adhesion with diminished expression of E-cadherin. E-cadherin, a calcium-dependent transmembrane glycoprotein expressed in most epithelial tissues, constructs a tight junction which connects adjacent cells. The loss of E-cadherin can lead to tumor progression, metastasis, and poorer prognosis in various human carcinomas.[10,11,24,25] Genetic or epigenetic alterations cause a functional loss of E-cadherin. For instance, mutations in E-cadherin are found in diffuse gastric cancer[26] and lobular breast carcinoma.[27] In addition, hypermethylation of the E-cadherin promoter region is found in various human carcinomas, resulting in frequent loss of E-cadherin expression.[28,29] Interestingly, Graff *et al.* proposed that the degree of methylation of the E-cadherin promoter region during metastatic progression is unstable and heterogeneous.[28] This finding suggests that the loss of E-cadherin by methylation in a primary lesion may drive metastatic progression, indicating that EMT is involved in cancer metastasis. Besides genetic or epigenetic control, E-cadherin is regulated by various signal networks, such as TGF-β signaling and transcription factors as discussed in more detail below.

TGF-β signaling. Miettinen *et al.* first revealed that TGF-β induced EMT in normal mammary epithelial cells.[30] In fact, TGF-β is an important inducer of EMT in cancer progression. However, TGF-β is well known to induce multiple responses in cancer progression.[31] For example, loss of the TGF-β signaling pathway results in the progression of cancer because TGF-β is a strong growth inhibitor.[32] Indeed, Hahn *et al.* reported that mutations in TGF-β and Smad4 give rise to pancreatic cancer[33] and colorectal cancer.[34] On the other hand, TGF-β can protect against apoptosis, and promote angiogenesis and immune suppression.[35] TGF-β induces EMT through multiple signal pathways, including direct phosphorylation of Smad 2 and Smad 3. As shown in Figure 1, TGF-β also activates other EMT-related signal pathways, including integrin, Notch, and Wnt signal pathways, all of which trigger EMT programs.

Transcription factors. Transcriptional repressors of E-cadherin such as zinc finger proteins (ZEB1, ZEB2), bHLH protein (Twist), and the snail family of zinc finger proteins (Snail, Slug) are associated with EMT.[36–40] As shown in Figure 1, various signal pathways such as TGF-β,[20] the Wnt cascade, and PI3K/AKT (phosphatidylinositol 3′ kinase–serine/threonine kinase) axis are connected with these transcriptional repressors of E-cadherin.[41] Recent studies have demonstrated that transcriptional repressors of E-cadherin are regulated by microRNAs as described below. Several transcriptional factors such as Snail, Slug, and Twist are useful markers to predict prognosis in various human carcinomas (Table 1). Peinado *et al.* proposed that E-cadherin repressors might participate in the process of EMT as follows. First, Snail and ZEB2 would initiate down-regulation of E-cadherin. Then, Slug and ZEB1 would maintain repression of E-cadherin.[42] However, the effect of E-cadherin repressors on mesenchymal markers such as vimentin and N-cadherin remains unsolved.

Regulation of EMT by microRNA. Recent studies of small non-coding RNAs are shedding light on the regulation of gene expression and proteins in metastasis. It was shown that miR-10b overexpression is associated with invasiveness and metastatic potential.[43] miR-10b is overexpressed in metastatic breast cancer, and up-regulated by EMT transcription factor Twist. Recent independent studies revealed that the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) and miR-205 play critical roles in regulating EMT, targeting the E-cadherin repressors ZEB1 and ZEB2.[13,15] Gibbons *et al.* found that metastasis-prone tumor cells established from

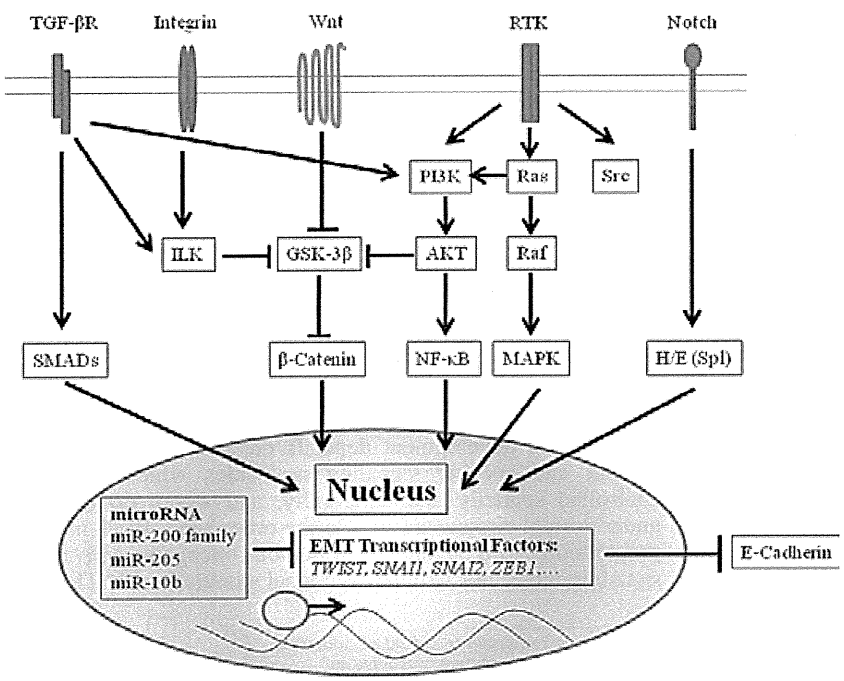

Fig. 1. Depiction of signal pathways regulating the epithelial–mesenchymal transition (EMT). Selected signal pathways regulating E-cadherin are schematized. Transforming growth factor (TGF)-β signals toward the SMAD pathway or the PI3K/AKT axis. Wnt ligands block β-catenin degradation. Excess β-catenin enters the nucleus and upregulates *SLUG* and *SNAIL* transcription. In integrin signaling, overexpression of ILK leads to nuclear translocation of β-catenin. Signals via RTK lead to EMT through the Ras-Raf-MAPK pathway or the PI3K/AKT pathway. AKT, serine/threonine kinase; GSK-3β, glycogen synthase kinase-3β; H/E (Spl), Hairy and enhancer of split; ILK, integrin-linked kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3′ kinase; RTK, receptor tyrosine kinase; TGF-βR, transforming growth factor-β receptor.

doi: 10.1111/j.1349-7006.2009.01419.x