criterion to avoid underestimating gefitinib effectiveness. CEA has been reported as a useful clinical therapeutic marker.[19] When the elevated CEA level decreased to a level less than half of the baseline level, gefitinib treatment was judged as effective. On the other hand, gefitinib treatment was judged as ineffective when the tumors showed any growth or a new lesion appeared in the imaging studies, or when the serum CEA level increased. Any patient who did not fit either of these criteria was classified as not assessable. All these evaluations were done before the *EGFR* gene analysis, without knowledge of mutational status of the *EGFR* gene.

Statistical Analysis

For comparisons of proportions, the χ^2 test or Fisher's exact test was used. The Kaplan-Meier method was used to estimate the probability of survival as a function of time, and survival differences were analyzed by the log-rank test. The two-sided significance level was set at $P < .05$. To identify which independent factors had a joint significant influence on gefitinib effectiveness, the logistic regression modeling technique was used, and for multivariate analysis of the overall survival, the Cox proportional hazards modeling technique was applied. All analyses were performed using StatView version 5 (SAS institute Inc, Cary, NC) software on a Macintosh computer.

RESULTS

EGFR *Mutations*

Mutations of the *EGFR* gene were detected in 33 (56%) of 59 patients. Seventeen were deletions, 15 were point mutations, and one was an insertion. Details of these mutations are shown in Figure 1. As previously reported,[15-17] *EGFR* mutations were significantly associated with adenocarcinoma histology, female sex, and never-smoking status (Table 1). However, the mutations were not associated with the age or stage of the patients. Furthermore, median time from the original surgery to

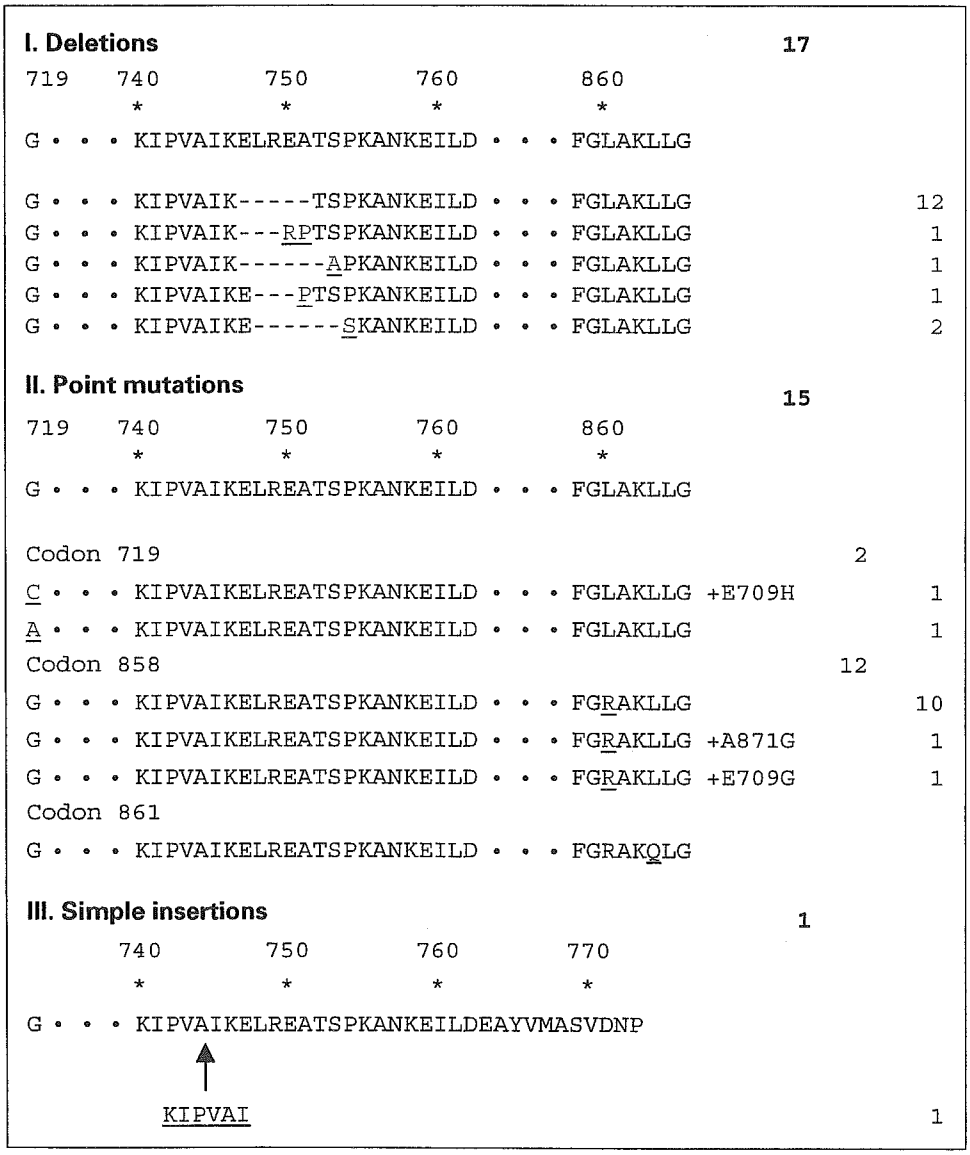

Fig 1. Analysis of 33 epidermal growth factor receptor (EGFR) mutations in tyrosine kinase domain of the *EGFR* gene found in unselected patients with lung cancer.

Table 1. Incidence of EGFR Mutations and Clinical and Pathologic Features

Variable	EGFR Mutation No. of Patients	%	EGFR Wild-Type	P
All cases	33	56	26	
Sex				
Male	14	44	18	.0402
Female	19	70	8	
Age, years				
≤ 64	22	55	18	.8342
> 64	11	58	8	
Histologic type				
Adenocarcinoma	32	64	18	.0033
Nonadenocarcinoma	1	11	8	
Squamous cell carcinoma	0	0	5	
Large-cell carcinoma	0	0	3	
Adenosquamous carcinoma	1	100	0	
Smoking status				
Never smoker	20	71	8	.0227
Former or current smoker	13	42	18	
Stage				
I-II	12	50	12	.4472
III-IV	21	60	14	

Abbreviation: EGFR, epidermal growth factor receptor.

recurrence was almost identical in patients with *EGFR* mutations (362 days) and in those without *EGFR* mutations (363 days; $P = .8265$).

Clinical Improvement After Gefitinib Treatment

Forty-one of 59 patients had measurable disease at recurrence with imaging studies. Of these, 20 showed appreciable tumor shrinkage after gefitinib treatment, whereas 17 tumors increased in size, and there was no change in tumor size in four patients. All of these 20 tumors (pulmonary metastases in 11, pleural disseminated nodules in two, hepatic metastases in two, mediastinal lymph node swelling in two, brain metastases in two, and chest wall tumor in one) showed at least a 30% decrease in diameter. Figure 2 shows representative imaging studies. A computed tomography scan of the chest in patient L703 (73-year-old woman, adenocarcinoma) showed masses in the right-lower lobe and marked improvement 8 weeks after gefitinib initiation. A computed tomography scan of the liver in patient L1492 (52-year-old woman, adenocarcinoma) showed masses in the right lobe of the liver and dramatic improvement 10 days after gefitinib initiation. A large chest-wall mass in the left back of patient L1362 (62-year-old man, adenosquamous carcinoma) before gefitinib treatment almost disappeared 13 weeks after gefitinib initiation. A left-lung tumor in patient L1171 (70-year-old woman, adenocarcinoma) was smaller 6 weeks after gefitinib initiation.

CEA was above the upper normal limit (5 ng/mL) at baseline in 32 patients. Serum CEA level decreased to $< 10\%$, $< 50\%$, and to $> 50\%$ of the baseline level in three, 12, and five patients, respectively, whereas CEA level increased in 12 patients. When we combined the results of imaging studies with CEA and judged according to our criteria, gefitinib treatment was effective in 26 (52%), not effective in 24 (48%), and not assessable in nine patients (Table 2). There was a good correlation between these two examinations. The imaging studies and change in CEA levels did not conflict in any patients. In 17 patients with measurable diseases and whose baseline CEA level was elevated, the CEA level decreased in all 11 patients showing tumor shrinkage and increased in all five patients showing tumor growth, except for one patient whose tumors showed no change in size ($P < .001$, Fisher's exact test), supporting the validity of our criteria.

We searched for a relation between gefitinib effectiveness and various clinical and pathologic features (Table 2). Never-smokers and patients with adenocarcinoma had a significantly higher incidence of gefitinib effect. However, we could not detect significant difference in gefitinib sensitivity by sex or presence of prior chemotherapy, probably because of the small sample size, although there was a trend that female and chemotherapy-naïve patients were more responsive.

Relationship Between Clinical Response to Gefitinib Treatment and EGFR Mutations

The incidence of *EGFR* mutations in terms of response to gefitinib treatment as judged by imaging studies and CEA levels is shown in Table 3. Of 20 patients who showed tumor shrinkage, 19 (95%) had mutations of the *EGFR* gene. On the other hand, two (12%) of 17 patients whose tumors grew after gefitinib treatment harbored *EGFR* mutations ($P < .001$, Fisher's exact test). In Figure 2, patient L703, L1492, and L1362 had *EGFR* mutations (delE746-A750, L858R, and E746-S752insA, respectively). Of three, 12, and five patients whose CEA level decreased to less than 10%, less than 50%, and to more than 50% of the baseline level after gefitinib treatment, three (100%), 10 (83%), and four (80%) had *EGFR* mutations, respectively. On the other hand, of 12 patients whose CEA level increased, three (25%) had *EGFR* mutations ($P = .004$, Fisher's exact test).

When we used our criteria combining the results of imaging studies with CEA, gefitinib was effective in 24 (83%) of 29 patients with *EGFR* mutations, whereas it was effective only in two (10%) of 21 patients without *EGFR* mutations ($P < .0001$; Table 2). There were three patients with *EGFR* mutations (two with L858R and one with G719A) whose CEA level increased after gefitinib treatment but did not have measurable diseases. There were also two patients with *EGFR* mutations, one with L858R+E709H and one with I744-K745 ins KIPVAI whose tumor progressed.

Logistic regression analysis (Table 4) showed that *EGFR* mutation was the only significant factor contributing to gefitinib sensitivity.

On the other hand, patient L1171, who showed a decrease in size of multiple pulmonary metastatic nodules

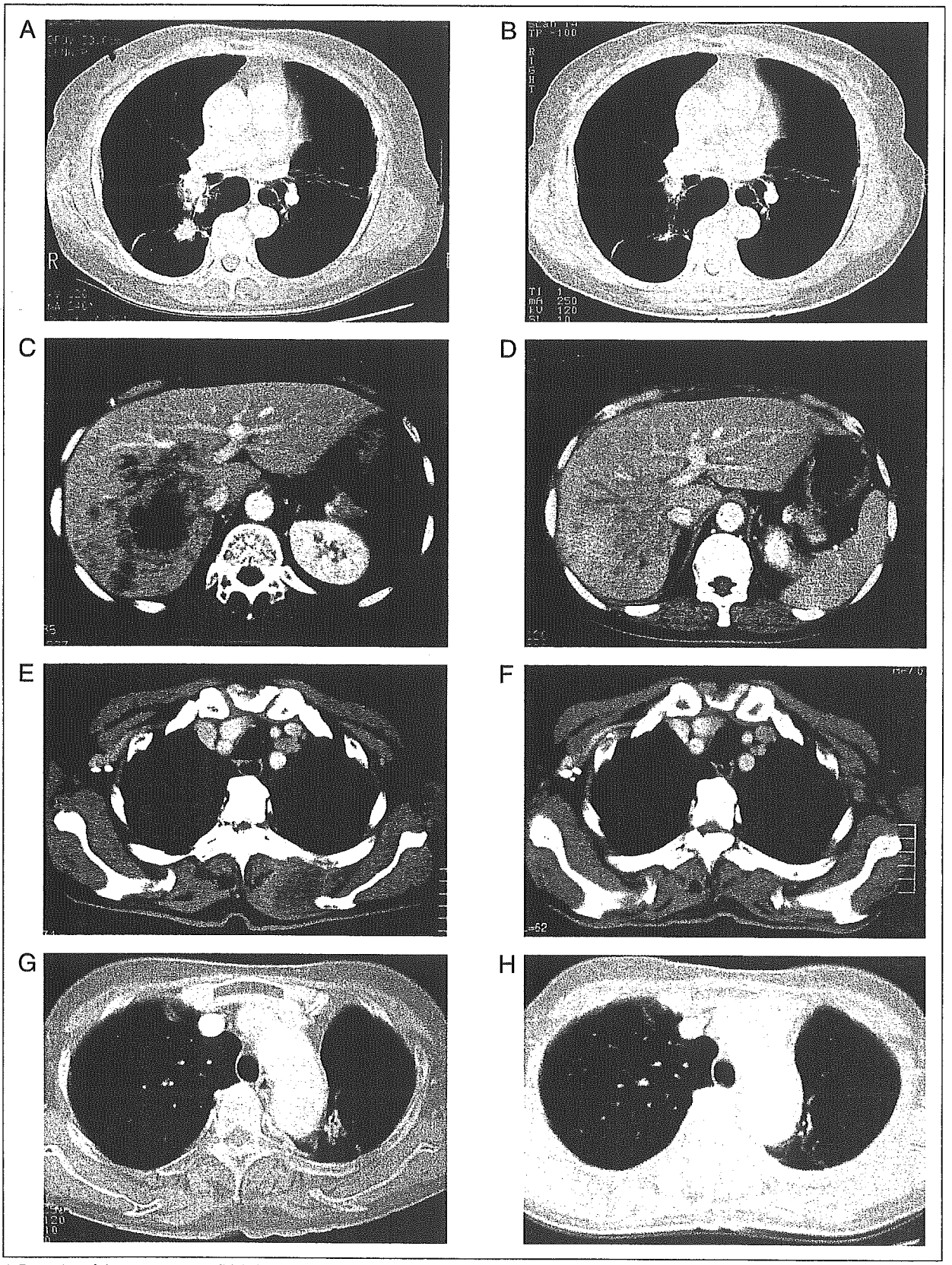

Fig 2. Examples of the response to gefitinib in representative four patients with recurrent non–small-cell lung cancer. Computed tomography (CT) scans before gefitinib treatment (A, C, E, G) and after the gefitinib was initiated (B, D, F, H) are shown. CT scans of patient L703 (A, B), patient L1492 (C, D), patient L1362 (E, F), and patient L1171 (G, H).

Table 2. Relation Between Gefitinib Effectiveness and Various Clinical and Pathologic Features

Variable	Effective: No. of Patients	Effective: %*	Not Effective	Not Assessable	P†
All patients	26	52	24	9	
Sex					
Male	11	41	16	5	.0842
Female	15	65	8	4	
Smoking status					
Never-smoker	17	68	8	3	.0235
Former or current smoker	9	36	16	6	
Histologic type					
Adenocarcinoma	25	58	18	7	.0313
Nonadenocarcinoma	1	14	6	2	
Prior chemotherapy					
Present	17	47	19	4	.2782
Absent	9	64	5	5	
EGFR mutation					
Mutation	24	83	5	4	< .0001
Deletion	16	100	0	1	.0108‡
Insertion	0	0	1	0	
Point mutation	8	67	4	3	
Wild-type	2	10	19	5	

Abbreviation: EGFR, epidermal growth factor receptor.
*Percentages were calculated excluding patients who were not assessable.
†*P* values were calculated excluding patients who were not assessable.
‡*P* value for Fisher's exact test comparing deletion mutants with the other mutants.

(Figs 2G and H) and a decrease in CEA level from 16.8 to 4.3 ng/mL, did not have *EGFR* mutations. In this patient, we extended our search for mutations to exons 22 and 23 of the *EGFR* gene, and still found none. Another patient without *EGFR* mutation in whom gefitinib was effective was a 59-year-old man who showed a decrease in serum CEA level from 10.6 to 1.5 ng/mL after 2 weeks of gefitinib treatment; this low level of CEA was maintained at least for 7 months.

When we further analyzed gefitinib response by classes of *EGFR* mutation, we found that there was a difference of response between patients with deletion mutations and those with the other types of mutations. Gefitinib was effective in all 16 patients with deletions, and effective in eight of 13 with other types of mutation ($P = .0108$).

Effect of EGFR *Mutation on Patient Survival After Gefitinib Treatment*

Patients with *EGFR* mutations survived for a significantly longer time calculated from the day of gefitinib initiation than those without *EGFR* mutations ($P = .0053$, log-rank test; Fig 3). Likewise, 26 gefitinib responders survived for a longer time than 24 nonresponders ($P = .0320$, log-rank test; not shown). Multivariate analysis revealed that *EGFR* mutation was the only factor that significantly and independently affected overall survival (Table 5). *EGFR* mutation class did not affect overall survival (not shown).

DISCUSSION

Recurrence after complete resection of NSCLC often presents as a form of distant metastases.[20] In clinical practice, chemotherapy is given to these patients except for a small number in whom re-resection of the tumor is indicated. Many studies have shown that chemotherapy prolongs survival and improves quality of life in unresectable stage IV tumors.[21] However, patients with unresectable tumors and patients with recurrent diseases may not be the same. There have been no large-scale randomized clinical trials addressing whether chemotherapy improves survival of patients with recurrence. Yoshino et al[22] found that chemotherapy for recurrence only tended to prolong survival in 118 of 468 consecutive patients who had recurrence after pulmonary resections. After introduction of gefitinib to clinical practice in 2002 in Japan, some patients with recurrent disease showed dramatic responses to gefitinib treatment, but many others did not respond. It has been unclear which patients respond to gefitinib and also whether gefitinib treatment prolongs survival in these patients.

Recent studies have showed striking correlation between gefitinib sensitivity and *EGFR* mutations both in vitro and in clinical studies.[15-17] Because this study was a retrospective analysis of response to gefitinib prescribed as routine care, judgment of gefitinib effectiveness tended to be less strict than that in a prospective clinical trial. Yet, changes in serum CEA level never conflicted with imaging studies. We were able to confirm a relation between *EGFR*

Table 3. Response to Gefitinib Treatment in 59 Patients With Recurrent Disease

CEA Level	Imaging Results: Shrinkage	No Change	Not Measurable	Growth	Total
Decreased					
<10% of the baseline	**3 (3)**				3 (3)
<50% of the baseline	**6 (5)**	**1 (1)**	**5 (4)**		12 (10)
>50% of the baseline	**2 (2)**		*3 (2)*		5 (4)
Not assessable	**9 (9)**	*3 (1)*	*3 (1)*	12 (2)	27 (13)
Elevated			7 (3)	5 (0)	12 (3)
Total	20 (19)	4 (2)	18 (10)	17 (2)	59 (33)

NOTE. Numbers in bold indicate that gefitinib treatment resulted in clinical improvement in these patients; numbers with underlines indicate the treatment resulted in progression of the disease; numbers in parentheses show number of patients with EGFR mutations in each category; and italicized numbers indicate that gefitinib treatment could not be assessed.
Abbreviations: EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen.

Table 4. Logistic Regression Analysis of Various Factors That Predict EGFR Effectiveness

Variable	Odds Ratio	95% CI	*P*
Sex			
Male/female	1.139	0.130 to 9.953	.9063
Smoking status			
Never/former/current	1.496	0.165 to 13.535	.7202
Histologic type			
Adenocarcinoma/ nonadenocarcinoma	1.727	0.091 to 33.33	.7159
Prior chemotherapy			
Yes/no	0.427	0.060 to 3.027	.3948
EGFR mutation			
Mutant/wild-type	40.000	6.024 to 2750	< .0001

Abbreviation: EGFR, epidermal growth factor receptor.

mutations and gefitinib sensitivity in a slightly different clinical setting. We correlated *EGFR* mutations found in specimens taken at the time of surgery with response to gefitinib, often after several courses of cytotoxic chemotherapy for recurrent disease. Multivariate analysis revealed that *EGFR* mutation was the only independent predictor for gefitinib response among several allegedly contributing factors. As in previous studies, *EGFR* mutation was not a perfect predictor of gefitinib effectiveness.[15-17] Two patients without *EGFR* mutations showed response to gefitinib. It is not clear at this time whether *EGFR* mutations are present in other parts of the gene or whether mechanisms other than *EGFR* mutations govern sensitivity in these patients.

We found a significant difference in gefitinib sensitivity according to classes of *EGFR* mutations. All 16 patients with deletion mutants responded to gefitinib, compared with eight of 12 patients with other mutations (P = .0108). It is not clear whether this difference is based on differences in biologic activity of these mutant proteins.

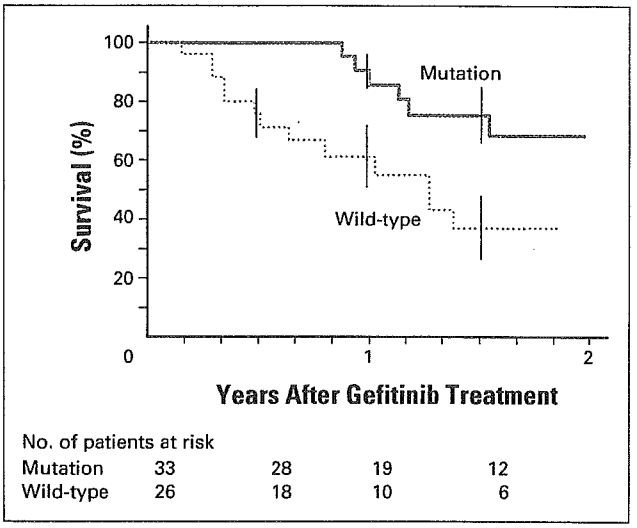

Fig 3. Effect of epidermal growth factor receptor mutations on survival, calculated from the day of initiating gefitinib treatment in patients who had recurrent disease after surgery (P = .0053, log-rank test).

Table 5. Cox Proportional Hazards Model for Survival Analysis

Variable	Hazard Ratio	95% CI	*P*
Sex			
Female/male	0.359	0.068 to 1.900	.2280
Smoking status			
Never/former/current	0.511	0.092 to 2.854	.4445
Histologic type			
Adenocarcinoma/ nonadenocarcinoma	0.335	0.095 to 1.184	.0894
Prior chemotherapy			
Yes/no	0.653	0.222 to 1.923	.4397
Stage			
I-II/III-IV	0.848	0.322 to 2.232	.7380
Age, years			
> 64/≤ 64	0.964	0.342 to 2.717	.9457
EGFR mutation			
Mutant/wild-type	0.342	0.117 to 0.998	.0496

Abbreviation: EGFR, epidermal growth factor receptor.

Gefitinib sensitivity was essentially the same in COS cells transfected with L858R and in cells transfected with del L747-P753insS.[16] A more recent study showed that the tyrosine residue at codon 845 is highly phosphorylated in L858R mutants, but not in deletion mutants after epidermal growth factor binding.[23] This might explain the difference in gefitinib response between tumors with L858R and those with deletions.

Although our criteria for tumor response are soft, these are merely a surrogate marker for the effect on survival. We were able to show, for the first time, that *EGFR* mutation was the only significant and independent predictor for a prolonged survival after gefitinib treatment. In a previous study, we showed that *EGFR* mutation itself is not a predictor for better postoperative survival in 236 unselected patients with adenocarcinoma,[24] and in the present study, median disease-free interval was almost identical in patients with or without *EGFR* mutations. A recent placebo-controlled clinical trial showed that treatment with erlotinib, another oral EGFR TK inhibitor, significantly prolongs survival after first and second chemotherapy for NSCLC,[25] although *EGFR* mutation frequency is reported to be around 10% in Western countries.[15-17] This result is interpreted to mean that a subset of patients without mutations have also benefited from erlotinib therapy. The present study suggests that if patients were selected by presence of *EGFR* mutations, it would be possible to concentrate patients with benefits from gefitinib treatment, avoiding unnecessary adverse reactions such as fatal interstitial lung disease, which is relatively common in Japanese patients.[26] Furthermore, our results provide a basis for postoperative adjuvant gefitinib treatment in NSCLC patients with *EGFR* mutations, as adjuvant treatment is considered the earliest treatment of metastatic disease. These possibilities should be tested in future clinical trials.

It is common for patients to show progressive disease soon after presenting an initial striking response to

gefitinib. However, we could not detect any evidence that differences in classes of *EGFR* mutations are associated with duration of response (data not shown).

In conclusion, tumors with *EGFR* mutations showed good, but not perfect, correlation with clinical response in patients with postoperative recurrence of NSCLC. Furthermore, patients with *EGFR* mutations survived for a significantly longer period than those without *EGFR* mutations. Future clinical trials using gefitinib should examine *EGFR* mutations for effective selection of patients who are most likely to benefit from this molecular-targeted drug.

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Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Honoraria: Tetsuya Mitsudomi, AstraZeneca Japan, Bristol-Myers Squibb Japan, TAIHO Pharmaceutical. For a detailed description of this category, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and Disclosures of Potential Conflicts of Interest found in Information for Contributors in the front of each issue.

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Reduced expression of *Dicer* associated with poor prognosis in lung cancer patients

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Emerging evidence suggests that microRNA, which are well-conserved, abundant and small regulatory RNA, may be involved in the pathogenesis of human cancers. We recently reported that expression of *let-7* was frequently reduced in lung cancers, and that reduced *let-7* expression was significantly associated with shorter patient survival. Two members of the double-stranded RNA-specific endonuclease family, *Dicer* and *Drosha*, convert precursor forms of microRNA into their mature forms using a stepwise process. In the present study, we examined expression levels of these genes in 67 non-small cell lung cancer cases, and found for the first time that *Dicer* expression levels were reduced in a fraction of lung cancers with a significant prognostic impact on the survival of surgically treated cases. It should be noted that multivariate COX regression analysis showed that the prognostic impact of *Dicer* ($P = 0.001$) appears to be independent of disease stage ($P = 0.001$), while logistic regression analysis demonstrated that the higher incidence of reduced *Dicer* expression in poorly differentiated tumors remained significant even after correction for other parameters ($P = 0.02$). Given the fundamental and multiple biological roles of *Dicer* in various cellular processes, our results suggest the involvement of reduced *Dicer* expression in the development of lung cancers, thus warranting further investigations of the underlying mechanisms, which can be expected to enhance understanding of the molecular pathogenesis of this fatal cancer. (*Cancer Sci* 2005; 96: 111–115)

Introduction

Lung cancer is the leading cause of cancer-related death in Japan, as it is in many other economically developed countries.[1,2] The mutation, amplification and epigenetic changes of various genes, which may eliminate the normal function of gene products, have been identified in lung cancers, suggesting that they may be involved in pathogenesis.[3] In addition, emerging evidence suggests that microRNA, which constitute a well-conserved and abundant class of approximately 22-nucleotide regulatory RNA, could also be involved. We previously reported that the expression of *let-7* was frequently reduced in lung cancers, both *in vitro* and *in vivo*, and that reduced *let-7* expression was significantly associated with shorter patient survival.[4] Furthermore, we were able to demonstrate that over-expression of *let-7* resulted in significant inhibition of *in vitro* growth of lung cancer cells. In addition to our findings in lung cancers, a number of studies have dealt with microRNA alterations in other types of human cancers. These alterations include down-regulation of *miR15* and *miR16* in chronic lymphocytic leukemia as well as of *miR-143* and *miR-145* in human colon cancers.[5,6] The biological functions of microRNA are not yet fully understood, but it has been suggested that they play a role in the coordination of cell proliferation and cell death during development, in addition to their involvement in stress resistance.[7–9] This evidence appears to lend support to the notion that microRNA alterations could be involved in the genesis and/or progression of various human cancers.

A double-stranded RNA (dsRNA)-specific endonuclease converts precursor forms of microRNA into mature forms through a stepwise process, which includes the generation of ~70nt pre-microRNA with a characteristic hairpin structure from the longer nascent transcripts (pri-microRNA), and further processing into its mature form.[7–9] In humans, *Dicer* and *Drosha* are thought to collaborate in this stepwise processing of microRNA, with *Drosha* executing the initial step of microRNA processing in the nucleus,[10] and the resultant premicroRNA being exported to the cytoplasm where they are cleaved by *Dicer* to generate the final products of ~22nt.[11–16]

In this study, we posed a question as to whether expressions of *Dicer* and *Drosha*, which are essential for the processing of microRNA, are altered in lung cancers, and whether changes in the expression have any effect on clinicopathological features. To this end, we examined 67 non-small cell lung cancer (NSCLC) cases, which had undergone potentially curative surgical resection, by means of real-time RT-PCR. We report here for the first time that the reduced expression of *Dicer* in a significant fraction of lung cancers was associated with shorter postoperative survival.

Materials and methods

Patients and tumor sample preparations. NSCLC samples were obtained from 67 patients who underwent potentially curative resection at the Aichi Cancer Center Hospital (Nagoya, Japan) between January 1996 and January 1998. Approval from the institutional review board and the patients' written informed consent were obtained. Stages were determined after pathologic evaluation of resected specimens according to the International System for Staging Lung Cancer, revised in 1997. The cohort consisted of 41 males and 26 females, with age at diagnosis ranging from 32 to 84 years (median age, 62 years). Thirty-seven patients had stage I disease, 13 patients stage II and 17 patients stage III. There were 15 patients with poorly differentiated, 43 with moderately and nine patients with well-differentiated tumors. Thirty-eight patients were smokers, and the remaining 29 had never smoked. A surgical pathologist (Y.Y.) performed a gross examination of the tissue specimens immediately after surgical removal, and pieces of tumor tissue were carefully selected for maximum tumor content. Half of each piece was snap frozen in liquid nitrogen, followed by storage at −80°C until use, and the other half was fixed with prechilled acetone and embedded in paraffin for confirmation of tumor contents. Total RNA was isolated by means of the standard acid

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guanidinium isothiocyanate/cesium chloride procedure using ultracentrifugation.

Relative quantification by real-time RT-PCR analysis. First-strand cDNA were synthesized from total RNA using Moloney murine leukemia virus reverse transcriptase (M-MLV RT) (Invitrogen, Carlsbad, CA, USA) and random hexamer primers (Roche Applied Science, Alameda, CA, USA). Real-time quantitative PCR amplification of a cDNA template corresponding to 20 ng total RNA was performed using SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) in an ABI PRISM 7900-HT (Applied Biosystems). PCR conditions were 50°C for 2 min, 95°C for 10 min, followed by 55 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 30 s. Standard curves were plotted by using serially diluted cDNA of the BEAS2B lung epithelial cell line, and the expression level of the samples was normalized with that of *18S rRNA* and expressed as a ratio of the normalized expression of the gene of interest in a mixture of the RNA of 38 normal lung tissues. Expression levels in this mixture of normal lung RNA were adopted as 1. The primer pairs used for *Dicer* were 5′-GTACGACTACCACAAGTACTTC-3′ and 5′-ATAGTACACCTGCCAGACTGT-3′, for *Drosha*, 5′-GTGC-TGTCCATGCACCAGATT-3′ and 5′-TGCATAACTCAACTGT-GCAGG-3′; and for *18S rRNA*, 5′-AATCAGGGTTCGATTCCGGA-3′ and 5′-CCAAGATCCAACTACGAGCT-3′.

DNA methylation analysis of *Dicer*. Extraction of genomic DNA from tissues was performed according to standard procedures. Genomic DNA were treated with the bisulfite conversion method as described in a previous study.[17] After conversion, the promoter region of *Dicer* was amplified by PCR and every CpG site within the region was examined with direct sequencing for the presence of DNA methylation. The primer sequence was designed on the basis of the converted sense strand sequences without CpG sites 5′-TTTATTTGGGTTTGTAGTAGT-3′ and 5′-AACCCTATCCAATCACAAACT-3′. The PCR mixture contained 1 unit of Platinum Taq DNA polymerase (Invitrogen) together with 1 × PCR buffer, 2.5 m*M* of $MgCl_2$, 25 pmol of each primer, and 0.2 m*M* of dNTP. PCR conditions were 95°C for 5 min, followed by 40 cycles at 95°C for 30 s, at 56°C for 30 s, at 72°C for 45 s, and at 72°C for 5 min. The PCR products were gel extracted (QIAquick Gel Extraction Kit; Qiagen, Valencia, CA, USA) and sequenced directly with the aid of an ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems).

Statistical analysis. The following biostatistical analyses were performed with the STATA statistical package release 7.0 (STATA, College Station, TX, USA). The χ^2 goodness-of-fit test was used to analyze whether the distribution of expression levels at $\log_2$ of *Dicer* or *Drosha* could be fitted to the normal distribution. Student's *t*-test was employed to determine the best cut-off value for separating two characteristic groups in terms of gene expression levels. The association between expression levels of *Dicer* and *Drosha* was analyzed by computing the Pearson correlation coefficient, and associations between various clinicopathologic characteristics and the expression levels of *Dicer* and *Drosha* were examined by means of Fisher's exact test. The Kaplan-Meier estimates of overall survival time were compared by using the log-rank test. Cox regression analysis of factors potentially related to survival was used to identify which independent factors might jointly have a significant effect on survival. All tests were two-tailed, and the significance level was set at $P < 0.05$.

Results

Reduced expression of *Dicer* in NSCLC. We used real-time RT-PCR analysis to examine 67 NSCLC cases, which had undergone potentially curative resection, for *Dicer* and *Drosha* expression. We found that there was a significant correlation between *Dicer* and *Drosha* expression in NSCLC, with a Pearson correlation coefficient of 0.79 ($P < 0.001$; Fig. 1a).

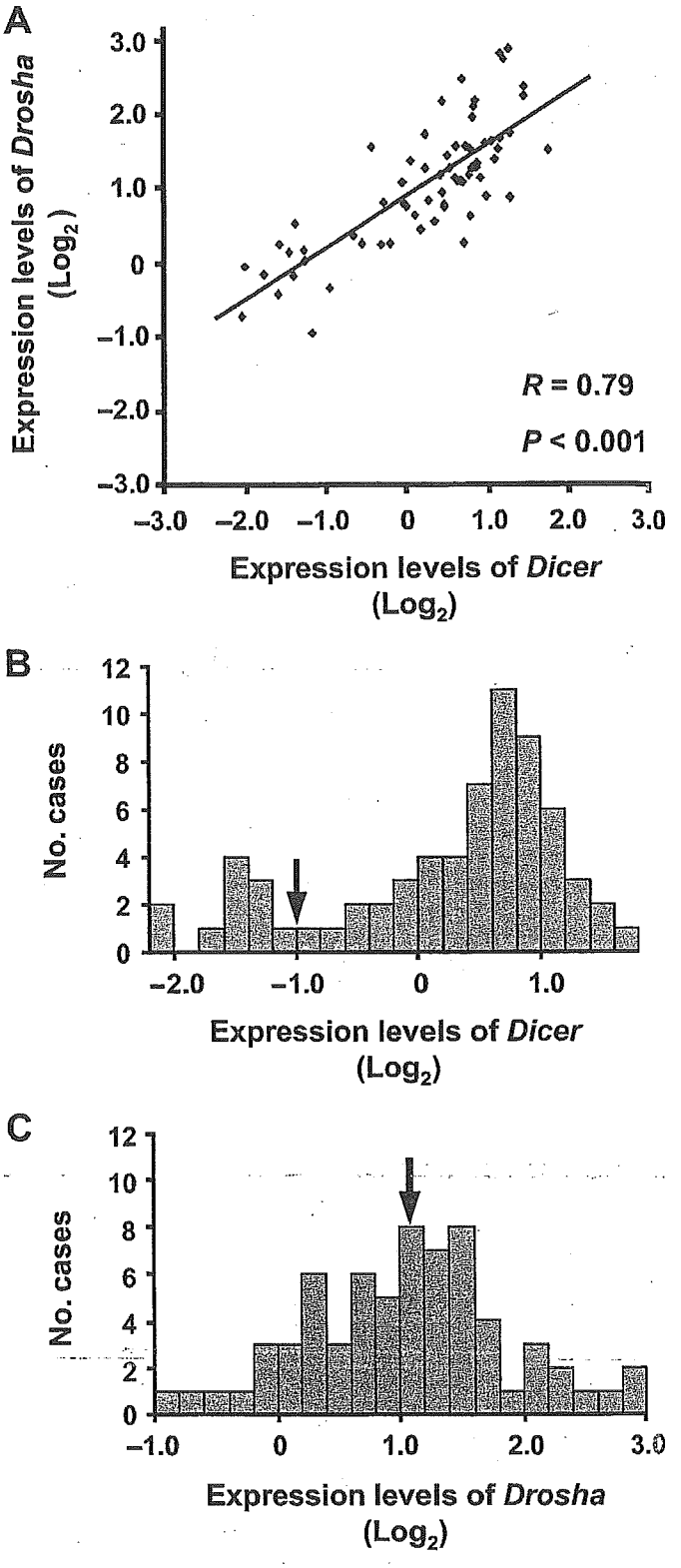

Fig. 1. Histograms of distributions of non-small cell lung cancers (NSCLC) according to their expression of double-stranded RNA-specific endonucleases mRNA. (A) Scattered plot analysis of expression levels of *Dicer* and *Drosha*. (B) Histogram of *Dicer* expression level at $\log_2$ value in NSCLC. With the threshold set at −1.0 of $\log_2$ value, patients were divided into two groups: low, with *Dicer* $\log_2$ value expression <−1.0; and high, with *Dicer* $\log_2$ value expression = −1.0. (C) Histogram of *Drosha* $\log_2$ value expression level in NSCLC. With the threshold set at 1.1 of $\log_2$ value, patients were divided into two groups: low, with *Drosha* $\log_2$ value expression <1.1; and high, with *Dicer* $\log_2$ value expression = 1.1. X-axis, $\log_2$ value; Y-axis, number of cases.

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able 1. Relationship between expression levels of *Dicer* and *Drosha* nd various clinicopathologic characteristics

haracteristics	No. cases	*Dicer* High	*Dicer* Low	*Dicer* P*	*Drosha* High	*Drosha* Low	*Drosha* P*
,ge (years)							
≤62	34	29	5	0.75	22	12	0.03
>62	33	27	6		12	21	
ex							
Male	41	34	7	1.00	21	20	1.00
Female	26	22	4		13	13	
listology							
Squamous	11	10	1	0.68	5	6	0.75
Non-squamous	56	46	10		29	27	
moking history							
Smoker	38	31	7	0.75	19	19	1.00
Non-smoker	29	25	4		15	14	
isease stage							
I	37	31	6	1.00	20	17	0.63
II-III	30	25	5		14	16	
ifferentiation							
Poor	15	9	6	0.01	5	10	0.15
Well or moderate	52	47	5		29	23	

Two-sided Fisher's exact test.

lowever, close inspection of the distributions of their expression isclosed a clear difference. A histogram of the expression of *)icer* showed a frequency distribution with two prominent eaks at $\log_2$ values from −1.6 to −1.4 and from 0.6 to 0.8 Fig. 1B), which was in marked contrast to that of *Drosha* Fig. 1C). We used the χ^2 goodness-of-fit test to determine vhether the observed frequency distributions of expression of *)icer* and *Drosha* could be fitted to the normal distribution. In he case of *Dicer*, it was clear that the data were not normally listributed ($P < 0.001$). The Student's *t*-test was therefore used o identify the cut-off value with the highest potential for liscriminating two distinct groups in terms of *Dicer* expression. 'atients could be divided most clearly and consistently into wo groups with low and high expression of *Dicer* when the listribution threshold was set at −1.0 of the $\log_2$ ratio of *Dicer* xpression. In contrast to the findings for *Dicer*, the hypothesis hat the distribution of *Drosha* follows a normal distribution attern could not be rejected ($P = 0.97$). Accordingly, the nedian expression level (i.e. 1.1 of the $\log_2$ value) was chosen s the threshold value to be used for further analysis.

Relationships between expression of *Dicer* and *Drosha* and various linicopathologic characteristics. Our next investigation was con-erned with whether expression levels of either *Dicer* or *Drosha* howed any relationship with the clinicopathologic charac-eristics of lung cancers, and found that there was a statistically ignificant association between *Dicer* expression levels and lifferentiation grade (Table 1). Cases with low *Dicer* expression howed significantly greater prevalence of poorly differentiated umors than those with high *Dicer* expression ($P = 0.01$), which vas also observed in the multivariate logistic regression analysis vith adjustment for all the variables analyzed in the univariate nalysis ($P = 0.02$).

Association between low *Dicer* expression and shortened ostoperative survival. The next question to be examined was vhether expression levels of *Dicer* and *Drosha* were associated vith patient survival after surgery. The Kaplan-Meier survival urves demonstrated that the probability of survival was ignificantly lower for the group of patients with low levels of *)icer* expression ($P = 0.0001$ by log-rank test; Fig. 2A), while ow expression of *Drosha* tended to be associated with a worse rognosis ($P = 0.06$ by log-rank test; Fig. 2B). Prognostic values f various factors were studied by univariate Cox regression

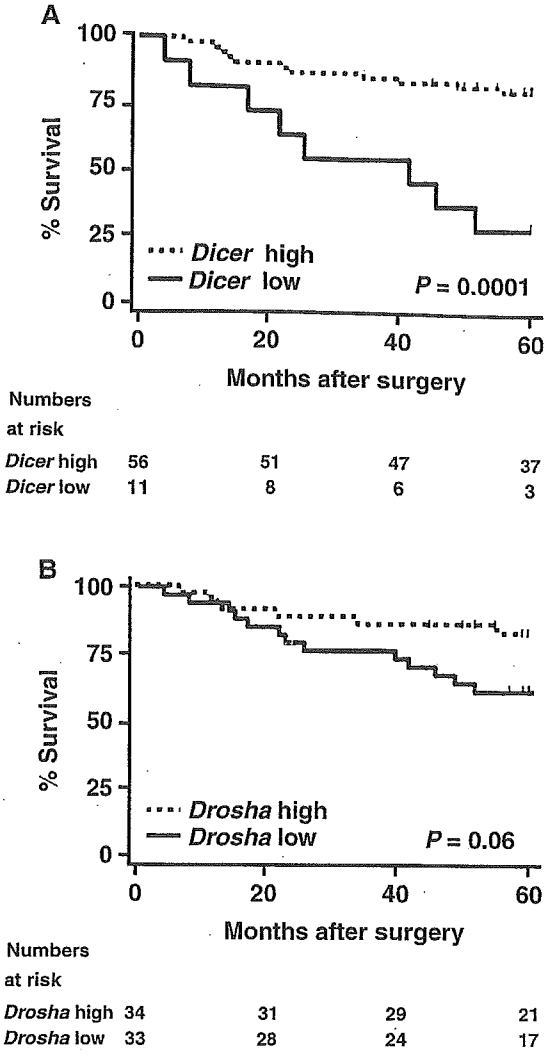

Fig. 2. Analysis of overall survival of patients with high or low expression of double-stranded RNA-specific endonucleases. (A) Kaplan-Meier survival curves for lung cancer patients, who were classified as showing either high or low *Dicer* expression. *Dicer* status was found to be strongly associated (log-rank, $P = 0.0001$) with patient survival. (B) Kaplan-Meier survival curve for lung cancer patients, who were classified as showing either high or low *Drosha* expression. *Drosha* status did not show a significant (log-rank, $P = 0.06$) relationship with patient survival. X-axis, length of survival after surgery; Y-axis, percentage of survivors.

analysis (Table 2). It was shown that, in addition to disease stage ($P = 0.003$), low *Dicer* expression was a significant predictive factor for poor prognosis ($P < 0.001$), whereas the *Drosha* expression level did not show a significant association with survival ($P = 0.07$).

The interrelationship of possible prognostic factors and survival was further analyzed by means of the Cox proportional hazards modeling using age, sex, histology, smoking history, disease stage and differentiation as well as expression levels of *Dicer* and *Drosha* as variables. As a result, reduced expression of *Dicer*, in addition to disease stage ($P = 0.001$), was identified as a significant and independent prognostic factor ($P = 0.001$) for surgically treated NSCLC patients after potentially curative resection. The hazard ratio for earlier death was 17.6 [95% confidence interval: 3.49–89.1] for low versus high expression levels of *Dicer*. These findings provided a strong indication that the expression levels of *Dicer* appeared to have a significant impact on the postoperative survival of NSCLC patients.

Table 2. Univariate and multivariate Cox regression analyses of the relationship between expression levels of *Dicer* and various clinical characteristics

Univariate analysis			
Variables	HR [95% CI]	Unfavorable/Favorable	*P*
Age (years)	2.02 [0.80–5.14]	>62/≤62	0.14
Sex	2.79 [0.92–8.41]	Male/Female	0.07
Histology	1.37 [0.45–4.14]	Squamous/Non-squamous	0.58
Smoking history	2.52 [0.91–7.01]	Smoker/Non-smoker	0.08
Disease stage	4.61 [1.66–12.85]	II-III/I	0.003
Differentiation	2.55 [1.00–6.49]	Poor/Well or moderate	0.05
Dicer	5.18 [2.07–12.97]	Low/High	<0.001
Drosha	2.45 [0.93–6.45]	Low/High	0.07

Multivariate analysis			
Variables	HR [95% CI]	Unfavorable/Favorable	*P*
Age (years)	1.86 [0.64–5.43]	>62/≤62	0.26
Sex	1.08 [0.25–4.64]	Male/Female	0.92
Histology	1.14 [0.29–4.51]	Squamous/Non-squamous	0.85
Smoking history	2.89 [0.75–11.1]	Smoker/Non-smoker	0.12
Disease stage	11.3 [2.87–44.3]	II-III/I	0.001
Differentiation	0.48 [0.12–1.86]	Poor/Well or moderate	0.29
Dicer	17.6 [3.49–89.1]	Low/High	0.001
Drosha	0.91 [0.25–3.36]	Low/High	0.88

HR, hazard ratio; CI, confidence interval.

Lack of DNA methylation of the *Dicer* promoter region. Because DNA methylation of the promoter region is thought to be significantly involved in transcriptional regulation,[18] we used the bisulfite conversion technique to study DNA methylation of the *Dicer* promoter region in 15 NSCLC (10 with low *Dicer* expression and five with high *Dicer* expression), as well as in three normal lung tissues. No methylation of the *Dicer* promoter region was found in any of the cases regardless of the level of *Dicer* expression, thus suggesting the involvement of other underlying mechanisms in the reduction of *Dicer* expression.

Discussion

In the study presented here, we have shown that the reduced expression of *Dicer* in a significant fraction of lung cancers is associated with shorter postoperative survival. To the best of our knowledge, ours is the first report of alterations of *Dicer* in human cancers. It should be noted that among the variables used in the multivariate COX regression analysis (i.e. age, sex, histology, smoking history, disease stage and differentiation as well as expression levels of *Dicer* and *Drosha*), *Dicer* appears to have a significant prognostic impact ($P = 0.001$) independent of disease stage ($P = 0.001$). Because logistic regression analysis demonstrated that the higher incidence of reduced *Dicer* expression in poorly differentiated tumors remained significant even after correction for other parameters ($P = 0.02$), one can speculate that prognostic impact of poor differentiation may well be represented by the presence of reduced expression of *Dicer*. Although our finding needs to be confirmed, for example on the cutoff value of *Dicer* expression level, by a further validation study using an independent and larger cohort, reduced expression of *Dicer* appears to be clinically useful for the prognosis of lung cancer patients. As for the underlying mechanisms involved in reduced *Dicer* expression in lung cancers, our study suggests that the involvement of hypermethylation of CpG sites in the promoter region is unlikely, so that other possibilities such as altered chromatin conformation and haploinsufficiency need to be pursued.[18] Corresponding to this, the frequent occurrence of loss of heterozygosity (LOH) on the long arm of chromosome 14, where *Dicer* resides, has been reported in lung cancers,[19,20] while a number of studies have also indicated that this chromosomal region is often affected in various other human cancers.[21–26] It is interesting that LOH on 14q appears to be related to tumor progression of colon cancer, with a higher incidence of this anomaly in metastatic sites than in primary tumors.[27]

Accumulating evidence supports the notion that the prognostic impact of reduced *Dicer* expression observed in our study might have a functional role in the development of lung cancers rather than being a mere surrogate marker. In correspondence with this, we recently reported that expression levels of *let-7* microRNA were frequently reduced in lung cancers, both *in vitro* and *in vivo*, and that lung cancer patients with reduced *let-7* expression had a significantly worse prognosis after potentially curative resection independent of disease stage.[4] We note that significant associations between reduced expression of *Dicer* and those of *let-7a-1* ($R = 0.66$, $P < 0.001$) and *let-7f-1* ($R = 0.65$, $P < 0.001$) were observed in this study. Since *Dicer* is required in the processing and generation of a fully mature form of microRNA,[11–16] it is not inconceivable that reduced *Dicer* expression may constitute an alternate post-transcriptional mechanism, which can also reduce expression levels of *let-7* and probably other microRNA in cancer cells.

In addition, other factors may underlie the potential biological effects of reduced *Dicer* expression in lung cancer cells. In fact, accumulating evidence suggests that the RNAi machinery may be functionally linked to the regulation of chromosome dynamics and genomic integrity. Furthermore, eukaryotic heterochromatin is characterized by a high density of repeats as well as by modified histones, and influences both gene expression and chromosome segregation. It was also found that deletion of *Dicer* in the fission yeast *Schizosaccharomyces pombe* resulted in the aberrant accumulation of complementary transcripts from centromeric heterochromatic repeats, loss of histone H3 lysine-9 methylation, and impairment of centromere function, resulting in defects in proper chromosome segregation.[28–32] The presence of marked aneuploidy is one of the key features of lung cancers,[33] while we previously reported the presence of a persistent increase in the rate of chromosomal losses and gains (i.e. chromosome instability, or CIN),[34] as well as of frequent impairment of mitotic checkpoints in lung cancer cell lines.[35] Therefore, the results of the present study raise the possibility that reduced *Dicer* expression in lung cancers may render cancer cells susceptible to chromosomal missegregation, in part because of the dysfunction of centromeres in the absence of a surveillance mechanism, which is the impairment of mitotic checkpoints.

It has also been suggested that the RNAi machinery might be involved in X inactivation and imprinting through sequence-specific histone modification and consequential DNA methylation and epigenetic silencing.[28] Therefore, it is possible that reduced expression of *Dicer* may affect such transcriptional regulation resulting from the altered activity of the RNAi machinery. In this connection, it should be noted that we previously found that loss of genomic imprinting is a frequent event in human lung cancers.[36,37] It would therefore be of considerable interest to study the involvement of a reduction in *Dicer* expression in relation to altered genomic imprinting in lung cancers.

In conclusion, we have been able to demonstrate for the first time that *Dicer* expression levels are reduced in some lung cancers with a significant prognostic impact on the survival of surgically treated cases. Given the fundamental and multiple biological roles of *Dicer* in various cellular processes, our results suggest the involvement of reduced *Dicer* expression in the development of lung cancers, and clearly warrant further investigations of the underlying mechanisms by which this alteration affects patient prognosis for a better understanding of the molecular pathogenesis of this fatal cancer. In addition, future studies to investigate

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whether altered *Dicer* expression is present in other types of human cancers should be both interesting and important.

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総 説

がん検診の有効性評価：新たなガイドライン作成にむけて

Evaluation of cancer screening programs for the development of new guidelines

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Abstract

To promote cancer screening programs correctly, evidence-base guidelines are absolutely necessary. In Japan, guidelines (the so called Hisamichi report) have been developed since 1998 by a research group funded by the Ministry of Health, Labour and Welfare. Organized screening based on their evidence has been conducted in municipalities nationwide. Although opportunistic screening using various modalities is performed in the clinical setting, it includes the modalities which effects are unclear.

The development of guidelines should be based on a standardized method. The indicator of evaluation of the efficacy of a cancer screening program is reduction of the mortality from a specific cancer but not the detection or survival rates. Two types of evidence are used for evaluation. Direct evidence is the result obtained from the studies examining effectiveness in reducing mortality, e.g., randomized controlled trials, cohort studies, and case-control studies. On the other hand, since indirect evidence, is insufficient to prove the reduction of mortality, the accumulation of two or more studies based on an analytic framework is necessary. For example, the data concerning sensitivity and specificity, distribution of detected cancer and the survival rate are applicable to indirect evidence. The evaluation of harm is also needed. Recommendation is determined based on the level of evidence and harm. In new guidelines for cancer screening, modalities of a specific cancer screening program are recommended to be conducted as either organized or opportunistic screening. The procedure for guideline development was fixed and applied to the revision of the guideline for colorectal cancer screening by a new research group funded by the Ministry of Health, Labour and Welfare. Further revisions of guidelines for other cancer screening programs are now being planned based on adequate assessment.

Key Words: cancer screening, evaluation, mortality reduction

1. 有効性評価の必要性

がん検診が公共政策として実施されるためには，がんによる死亡抑制についての科学的根拠を検証する必要がある。昭和58年の老人保健法施行以来，がん検診はわが国の公共政策として実施されている。一方，「がん検診」と称されて行われている検診のなかには，適切な科学的根拠のない方法もある。科学的根拠とは，確立された研究方法により，その有効性が証明されたということを意味する。国民全体の健康を改善するための公共政策の目的から考えると，有効性の不明ながん検診を行うことは，わが国に大きな損失を及ぼすものになりかねない。国際的にもがん検診の有効性を評価し，公共政策に活用するという動きがあり，米国予防サービス特別委員会（U.S. Preventive Services Task Forces），カナダ予防医学特別委員会（Canadian Task Forces on Preventive Health Care）が予防対策の評価を行い，その成果を公表している。こうした流れを受け，わが国でも平成10年，11年，13年と過去3回にわたるがん検診の有効性評価が行われた。その第3回目

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が，平成13年3月に公表された平成12年度厚生労働省老人保健事業推進費等補助金　がん検診の適正化に関する調査研究事業「新たながん検診手法の有効性の評価」報告書（主任研究者 久道 茂）である[1]。

近年，欧米では，臨床ガイドラインを巡る研究が活発化しており，政策決定にも広く利用されている。2001年にはECを中心としてAGREE（Appraisal of Guideline for Research and Evaluation)[2,3] が発足し，引き続き，GIN (Guidelines International Network)[4]，COGS(Conference on Guideline Standardization)[5,6]，GRADE（Grade of Recommendation, Assessment, Development, and Evaluation）Working Group[7] により，臨床ガイドラインの標準化や推奨基準の見直しが提唱され，ガイドライン作成についての国際共同研究が進められるようになった。今後，わが国においても，諸外国の動向を見据え，最新の知見に基づき，有効性評価の継続的な更新を行い，政策決定の基礎資料を提供する必要がある。

2.「発見率」「生存率」による評価の限界

がん検診の評価方法として，通常よく用いられるものとして「発見率」や「生存率」がある。これらは多くの施設からの報告があること，算出が容易であること，また両者とも臨床医をはじめとする医療従事者になじみやすい指標であることから，広く用いられている。しかし，両者共，がん検診の有効性を適切に示す指標でない。これらの指標は，検診の有効性を真に評価することが困難となるバイアスを含んでいる。バイアスとは偏りのことで，真の状況からはかけ離れた状態を示している。

「発見率」はスクリーニング方法の精度だけでなく，対象となる集団の有病率の影響を受ける。がんの罹患は年齢が高くなるほど，特に60歳以上では急激に増加する。たとえば，胃がんでは60歳以上の受診者が多い地域検診では発見率が高く，30～40歳代が中心の職域検診では発見率が低くなる。発見率の差は，がん検診の方法の精度や診断能力の差よりも対象となる集団の年齢や性別に大きな影響を受ける。

さらに，「発見率」に影響する要因としては，受診者の検査歴がある。発見率は，初回の受診者では高く（prevalence screen），前年の検査歴がある逐年検診の場合は低い（incidence screen)。両者の差は，発見可能ながんがどのくらいあるかという滞在時間（sojourn time）に影響を受ける（図1)。滞在時間は前臨床期に相当し，この間に特定の検査を受けることで，がんが発見される可能性がある[8]。この滞在時間が長ければ長いほど，発見できるがんが多くなり，感度が多少低くても，発見率は高くなる。一方，前年の検査歴がある場合には発見可能ながんは，追加の1年に新たに発生したがんに限定される。胃間接X線法とペプシノゲン法の感度を同時法で比較した研究では，ペプシノゲン法の感度が高いとする報告が多い[9]。しかし，Tsubonoらも指摘しているように，これらの研究では，prevalence screenとincidence screenを同一に比較している。両者の感度を正確に行うためには，検査の既往を考慮した上で検討されなくてはならない。

一方，「生存率」にはがん検診特有のバイアスが紛れ込む可能性がある。そのバイアスが，リードタイム・バイアスやレングス・バイアスである。リードタイム・バイアスは，がんの成長や進展に関与するもので，検診によって発見された患者は有症状のために外来を受診した患者に比べ，がん発見が早いことから，見かけ上生存率が増加することで生じる。また，レングス・バイアスは，検診は成長のゆっくりしたがんを見つけやすく，有症状で発見される外来患者のがんに比べ予後が良くなる可能性を示している。

3. がん検診の精度

がん検診の精度を示す適切な指標としては，感度・特異度がある。感度は対象となる疾患を有する者が陽性となる割合である。図2では，感度はa/(a+b）となる。一方，特異度は対象となる疾患の

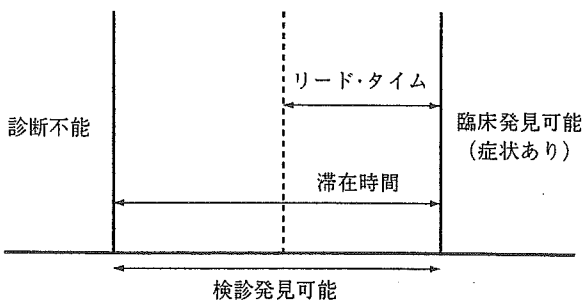


図1　検診発見可能がんと滞在時間

	がん(+)	がん(−)	
TEST(+)	a	c	a+c
TEST(−)	b	d	b+d
	a+b	c+d	a+b+c+d

感度＝a/(a+b)　　特異度＝d/(c+d)
陽性反応適中度＝a/(a+c)
陰性反応適中度＝d/(a+d)

図2　スクリーニングの精度

ない者の割合であり，d/(c+d) となる。感度は検査がどれくらい確実に対象となる疾患を拾い上げられるかを示しており，高いほど疾患を見逃さない。特異度は疾患のない者を識別するものであり，低い場合は疾患のない者が陽性となり（偽陽性），精密検査が増加することになる。検査をした場合，陽性となったもののうちどのくらいの割合で真に陽性であるか（疾患があるか）を示す指標として，陽性反応的中度がある。図2に従えば，陽性反応的中度は a/(a+c) で算出される。一方，陰性反応的中度は陰性になったうちどのくらいの割合で疾患がないかを示すもので，d/(b+d) で算出される。

感度と特異度は，がんのありなしを識別する検査の能力を示すものであり，対象となる集団の有病率に左右されない。しかし，陽性反応的中度や陰性反応的中度は対象集団の有病率に左右される。たとえば，感度も特異度も同等であっても，有病率の低い集団では，陽性反応適中度は低く，有病率の高いハイリスクグループや専門病院での陽性反適中度が高い。従って，陽性反適中度が高くても，地域住民を対象とした検診には使えない場合もある。

ただし，感度も定義によってその値は異なることから，様々な研究結果を単純に比較することはできない。感度の測定は同時法と追跡法により大別される。同時法は，同時に検査を行い，いずれかの検査で検出されたがんの総数を分母として，各検査の相対感度を算出するものである。どのような検査を組み合わせるか，また精密検査が確実に行われているかなどの要因が影響する。一方，追跡法による感度の測定は，追跡期間や中間期がんを把握するために，どのような方法がとられているか（がん登録や，本人・家族・主治医への問い合わせなど），またその捕捉状況が関与する。

4. 信頼性の高い検証方法（直接的証拠）

がん検診の有効性の評価には，最終的な健康結果である，死亡率や死亡数の減少を証明する必要がある。がんの「発見率」や「生存率」は，各種のバイアスが紛れ込むことから，単独でがん検診を評価することはできない。

有効性評価の方法として最も信頼性の高いのは，無作為化比較対照試験（Randomized Controlled Trial: RCT）である。次善の方法としては，コホート研究や症例対照研究があるが，その信頼性は下段に進むに従い低下する（表1）。

RCTはスクリーニングの対象となるがんの死亡率が非検診群に比べて検診群で低下するかを検証する試験である。がん検診の対象となる検診群と非検診群を無作為に割り付けることにより，両方の受診者の特性を近似させ，その上で検診を受けることにより，本当にがんによる死亡が減少するかを長期にわたって追跡する。

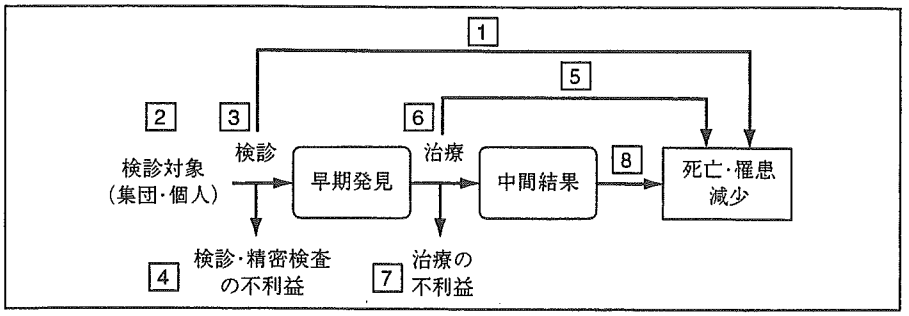
表1 有効性評価のための研究方法

1. 無作為比較対照試験
   (Randomized Controlled Trial: RCT)
2. コホート研究
3. 症例対照研究
4. 記述的研究
   横断研究
   症例報告
5. 専門家の意見

次善の方法として行われるコホート研究は検診受診群と検診非受診群を長期にわたって観察し，両者の死亡数を比較検討するものである。一方，症例対照研究は，がんの死亡者について，過去にがん検診を受診しているかどうかを調べ，症例群の死亡時に生存している集団から性や年齢を照合して抽出された対照群と比較検討する。コホート研究や症例対照研究ではセルフ・セレクション・バイアスが排除しにくく，バイアスの排除が可能なRCTに比べて結果の信頼性が高くなる。たとえば，家族歴や既往歴のある者が検診群に多い場合は，検診未受診者に比べ対象疾患の罹患率や死亡率が高くなることもある。また，健康の増進・保持に関心の高い人が検診受診者に多い場合，非受診者に比べ対象疾患の罹患率や死亡率が低い可能性がでてくる。こうした受診者の特性に関連するのが，セルフ・セレクション・バイアスである。

5. がん検診の有効性評価のための新たな方法（間接的証拠）

がん検診の有効性を評価するためには，死亡率減少効果を示す信頼性の高い研究が求められているが，これらの直接的証拠を示す研究を行うのは容易ではなく，時間を要する。このため，現存の証拠をいかし，評価に結びつける方法として，USPSTFではAnalytic Framework (AF) が導入された（図3）[10]。AFとは，検査や治療の結果を評価するために，検診，精密検査，治療の段階において，各段階における評価指標（検診によるる中間結果）を明確にし，最終的な結果である死亡率減少にどのように結びついていくかという，一連の流れとしてまとめたものである。AFは，各がん検診の特性を踏まえ作成し，各段階における検討課題を明らかにする。図3はい



各段階における検討課題

1. がん検診により，対象となるがんの死亡（あるいは罹患）を減少できるか
2. 対象集団における当該がんの罹患率（有病率）
3. 検診
   1) 精度（感度・特異度）はどの程度か。どのように算出されているか。他の検診方法と比較可能か。
   2) 発見がんの病期分布
4. 検診・精密検査の不利益
   1) 偽陰性・偽陽性
   2) 偶発症
   3) 過剰診断
   4) 受診者の負担
5. 適切な治療法が存在し，対象となるがんの死亡（あるいは罹患）を減少できるか
6. 適切な治療法が存在し，中間結果（進行がんなど）を減少できるか
   1) 治療効果の評価
   2) 検診発見がんと臨床がんとの生存率比較
7. 治療の不利益
   1) 偶発症
   2) 受診者の負担
8. 中間結果（進行がんなど）の減少が，対象となるがんの死亡（あるいは罹患）の減少につながるか

（祖父江友孝他：有効性評価に基づく大腸がん検診ガイドライン　普及版，2005）

図3　がん検診のAnalytic Frameworkと検討課題

表2　がん検診の評価に関する研究の現状と総合評価のまとめ

部位	検査法	検診発見がんと臨床診断がんの比較		死亡率減少効果				総合評価		
		進行度	生存率	無作為割付比較対照試験	無作為割付のない比較対照試験	コホート研究・症例対照研究	地域相関研究・時系列研究	評価判定		根拠の質
胃	胃X線検査	○	○	−	−	○	○		I−b	3
子宮頸部	頸部擦過細胞診	○	○	−	−	○	○		I−a	3
子宮体部	体部細胞診	○	○	−	−	−	−		II	−
乳房	視触診単独	○	○	○[b]	−	○	○	全年齢	I−c	3
	視触診+マンモグラフィ	○	○	○	○	○	○	50歳以上	I−a	1
								40歳代	I−b	1
肺	胸部X線+喀痰細胞診(日本)[c]	○	○	−	−	○	○		I−b	3
	胸部X線+喀痰細胞診(欧米)[c]	○	○	○	−	○	−		I−c	1
大腸	便潜血検査	○	○	○	○	○	○		I−a	1
肝	肝炎ウイルスキャリア検査	−	−	○	−	○	−		I−b	1

評価判定

I群　I−a　検診による死亡率減少効果があるとする，十分な根拠がある。
　　I−b　検診による死亡率減少効果があるとする，相応の根拠がある。
　　I−c　検診による死亡率減少効果がないとする，相応の根拠がある。
　　I−d　検診による死亡率減少効果がないとする，十分な根拠がある。

II群　現時点で，検診による死亡率減少効果の有無について判断する，適切な根拠がない。また，この中には，検査精度や生存率等を指標とする予備的な研究で可能性が示され，死亡率減少効果に関する研究が計画または進められているものを含む。

根拠の質

1　無作為割付比較対照試験
2　無作為割付のない比較対照試験
3　コホート研究と症例対照研究
4　地域相関研究と時系列研究
5　その他

がん検診の適正化に関する調査研究事業　新たながん検診の有効性評価報告書（主任研究者　久道茂）

ずれのがん検診においても共通となりうる過程と課題を示している。

間接的証拠となるのは，エンドポイントを発見がんなどの中間的結果に設定した研究や検査精度に関する研究が含まれる。AFの各段階を構成する検査精度（感度・特異度），発見がんの病期分布，生存率などの研究が該当する。これらは，個々の研究だけでは死亡率減少効果を証明することが困難だが，複数の研究の集積によりその効果が示唆されるものである。

6. がん検診の不利益

がん検診による最大の利益は，早期発見・早期治療によりがんから救命されることである。一方で，がん検診による不利益も存在する。偽陰性，偽陽性，過剰診断，偶発症，放射線被曝，感染，受診者の身体的・心理的負担などが，該当するが，その程度や重要性は各検査により異なる。

近年，諸外国の臨床ガイドラインにおいては，利益だけでなく，不利益を評価することが必須とされる。ただし，死亡率減少効果を証明するための評価とは異なり，不利益に関する評価は定まっていない。USPTSFでは，最終的判断となる推奨に，「利益が不利益をどの程度上回るか」（Net Benefit）が反映される[10]。一方，ガイドラインのチェックリストであるAGREEでは，数量的な評価ではなく，不利益を丁寧に記述したSIGNとNew Zeeland Guidelineの評価を望ましい評価例として取り上げている[11]。

7. 科学的根拠に基づく政策決定

平成13年3月に公表された久道班報告書第3版においては，老人保健事業によるがん検診の他，新たに行われている検診方法を含め，系統的総括を行い，根拠の質に基づき，評価判定を行った[1]。総合評価は，検査精度・検診発見がんと臨床診断がんの比較・死亡率減少効果・経済効果・不利益に関する個々の研究をまとめ，検診による死亡率減少効果の有無を判断するものである（表2）。さらに，死亡率減少効果の有無の根拠にも各々2段階に評価を行い，判断不能なものは保留（Ⅱ群）としている。根拠の質は，評価判定の根拠となった研究方法を示している。研究方法の妥当性は，死亡率減少効果を示す無作為化比較対照試験の評価が最も高く，死亡率減少効果に関する検討が行われていない場合には，5と判定されている。評価方法は主として死亡率減少効果を示す研究の研究方法に基づくものであり，他の要因については傍証的な位置づけとなっている。

わが国において，これまで行われてきたがん検診の評価は，研究班を主体としていたため，必ずしも定期的な評価の見直しや更新が予定されていたわけではない。また，久道班報告書第3版[1]はUSPSTF第2版[12]の手順を参考にし，評価判定が行われていたが，文献検索の方法，系統的総括の過程や推奨ルールは明確化されておらず，ガイドラインとしての体裁は十分とはいえなかった。諸外国では，公的常設機関により臨床ガイドラインの作成・更新が行われており，その詳細な手順も公表されている。今後，わが国においても，諸外国の動向を見据え，最新の知見に基づき，有効性評価の継続的な更新を行い，政策決定の基礎資料を提供する必要がある。

8. 新たなガイドライン作成にむけて

平成15年から，厚生労働省がん研究助成金「がん検診の適切な方法とその評価法の確立に関する研

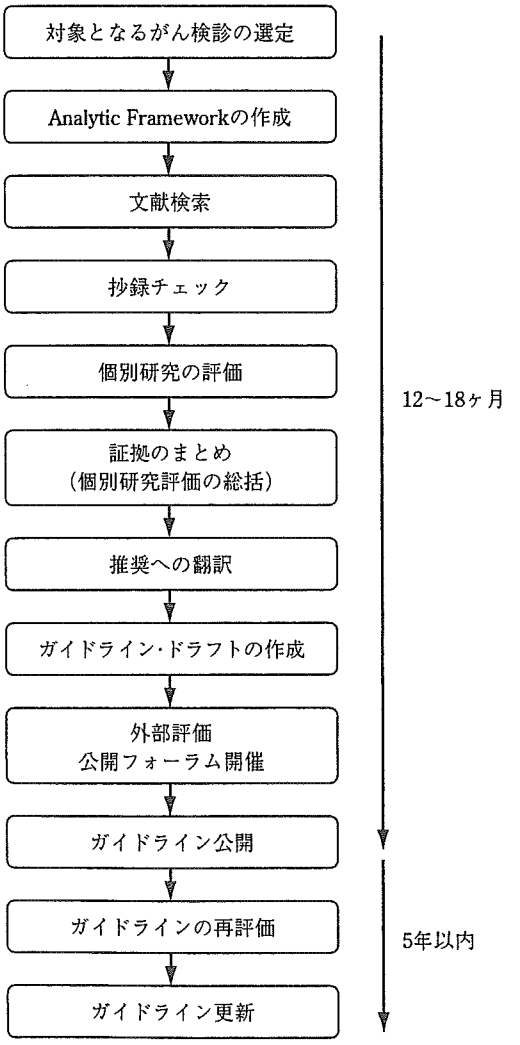


図4 ガイドライン作成過程
（祖父江友孝他：有効性評価に基づく大腸がん検診ガイドライン 普及版，2005）

究」班（主任研究者　祖父江友孝）（科学的根拠に基づくがん検診推進のページ　http://canscreen.ncc.go.jp/）では，がん検診ガイドライン作成手順の定式化に関する研究をすすめ，その手順に基づき，大腸がん検診ガイドラインを更新した[13]。

ガイドラインは，定式化された手順に従い，作成される（図4）[14]。がん検診による死亡率減少効果を明らかにするため，最新の知見も含めた対象文献の系統的総括を行い，各検診方法の直接的証拠と間接的証拠をまとめ，研究方法と質を反映した8段階に分類される証拠のレベルを決定する（表3）。不利益は，対象となるがん検診の特性を考慮し，各検診方法別の比較表に基づき検討される。その項目は，検診の種類により異なるが，偽陰性，偽陽性，過剰診断，偶発症，放射線被曝，感染，受診者の身体的・心理的負担などが該当する。死亡率減少効果と不利益に関する科学的根拠を示し，わが国において集団を対象とした対策型検診及び個人を対象とした任意型検診として実施の可否を5段階の推奨（表4）として総括される。更新された大腸がん検診ガイドラインの推奨のまとめは，表5に示した[13]。

正しくがん検診を推進していくためには，科学的根拠に基づくガイドラインが必要である。また，その評価方法も国際的に標準化された方法に基づくものでなければならない。しかし，不利益の評価や，推奨の決定，受診者の位置づけなど，未だ国際的にも議論もある課題も残っている。こうした動向を見据えながら，今後，他のがん検診についても，定式化された作成手順に基づき，ガイドラインの更新が行われる予定である。

表3　証拠のレベル

証拠レベル	主たる研究方法	内　容
1++	無作為化比較対照試験	死亡率減少効果の有無を示す，質が高く，バイアスの小さい無作為化比較対照試験が行われている
	系統的総括	死亡率減少効果の有無を示す，質の高いメタアナリシス等の系統的総括が行われている
1+	無作為化比較対照試験	死亡率減少効果の有無を示す，比較的質が高く，バイアスが小さい無作為化比較対照試験が行われている
	系統的総括	死亡率減少効果の有無を示す，比較的質の高いメタアナリシス等の系統的総括が行われている
	AF組み合わせ	Analytic Frameworkの重要な段階において無作為化比較対照試験が行われており，2++以上の症例対照研究・コホート研究が行われ，死亡率減少効果が示唆される
1−	無作為化比較対照試験	死亡率減少効果に関する質の低い，バイアスが大きい無作為化比較対照試験が行われている
	系統的総括	死亡率減少効果に関するメタ・アナリシス等の系統的総括が行われているが質が低い
2++	症例対照研究/コホート	死亡率減少効果の有無を示す，質が高く，バイアスや交絡因子が小さい症例対照研究・コホート研究が行われている
2+	症例対照研究/コホート	死亡率減少効果の有無を示す，中等度の質の，バイアスや交絡因子が小さい症例対照研究・コホート研究が行われている
	AF組み合わせ	死亡率減少効果の有無を示す直接的な証拠はないが，Analytic Frameworkの重要な段階において無作為化比較対照試験が行われており，一連の研究の組み合わせにより死亡率減少効果が示唆される
2−	症例対照研究/コホート	死亡率減少効果に関する，質が低く，バイアスや交絡因子が大きい症例対照研究・コホート研究が行われている
	AF組み合わせ	死亡率減少効果の有無を示す直接的な証拠はないが，Analytic Frameworkを構成する複数の研究がある
3	その他の研究	横断的な研究，発見率の報告，症例報告など，散発的な報告のみでAnalytic Frameworkを構成する評価が不可能である
4	専門家の意見	専門家の意見

AF: Analytic Framework

（祖父江友孝他：有効性評価に基づく大腸がん検診ガイドライン　普及版，2005）

表4 推奨のレベル

推奨	表　現	証拠のレベル
A	死亡率減少効果を示す十分な証拠があるので，実施することを強くすすめる。	1++/1+
B	死亡率減少効果を示す相応な証拠があるので，実施することをすすめる。	2++/2+
C	死亡率減少効果を示す証拠があるが，無視できない不利益があるため，集団を対象として実施することはすすめられない。 個人を対象として実施する場合には，安全性を確保すると共に，不利益について十分説明する必要がある。	1++/1+/2++/2+
D	死亡率減少効果がないことを示す証拠があるため，実施すべきではない。	1++/1+/2++/2+
I	死亡率減少効果の有無を判断する証拠が不十分であるため，集団を対象として実施することはすすめられない。 個人を対象に実施する場合には，効果が不明であることについて十分説明する必要がある。	1−/2−/3/4

注1) 集団を対象としたがん検診とは，集団の死亡率減少を目的として実施するものを示し，公共的な予防対策として行われる。
本ガイドラインは，集団を対象とした対策型検診と定義する。
市町村が行う老人保健事業による集団検診・個別検診や職域の法定健診に付加して行われる検診が該当する。
注2) 個人を対象とした検診とは，個人の任意により受診するがん検診を意味する。
本ガイドラインは，個人を対象とした任意型検診と定義する。
個人の死亡リスク減少を目的とし，対象となる個人は通常の診療の範囲外となる健常者である。
具体的には，検診センターや医療機関などで行われている総合健診や人間ドックなどに含まれているがん検診が該当する。

(祖父江友孝他：有効性評価に基づく大腸がん検診ガイドライン　普及版，2005)

表5 実施体制別大腸がん検診の推奨レベル

検診体制	対策型検診 Organized Screening	任意型検診 Opptunustic Screening
概要	集団全体の死亡率を下げるために対策として行う。	個人の死亡リスクを下げるために個人の判断で行う。
対象	集団	個人
具体例	老人保健事業による集団検診・個別検診 職域検診	人間ドック
スクリーニング方法	推奨	
便潜血化学法*1	○（推奨A）	○（推奨A）
便潜血免疫法*1	○（推奨A）	○（推奨A）
S状結腸鏡*2	×	○（推奨C）
S状結腸鏡＋便潜血化学法*2	×	○（推奨C）
全大腸内視鏡*2	×	○（推奨C）
注腸X線*2	×	○（推奨C）
直腸指診	×	×

*1 化学法に比べ，免疫法は，感度が高く，受診者の食事・薬剤制限を必要ないことから，免疫法を選択することが望ましい。
*2 無視できない不利益があることから，安全性を確保し，不利益について十分説明する必要がある。

(祖父江友孝他：有効性評価に基づく大腸がん検診ガイドライン　普及版，2005)

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がん検診の有効性評価：新たなガイドライン作成にむけて

濱島ちさと

わが国において正しくがん検診を推進していくためには，科学的根拠に基づくガイドラインが必要である。平成13年3月には，平成12年度厚生労働省老人保健事業推進費等補助金　がん検診の適正化に関する調査研究事業「新たながん検診手法の有効性の評価」報告書（主任研究者　久道茂）が公表された。市町村では同報告書に準じた検診が行われているが，検診機関などの任意型検診では効果の不明な多様な検診が行われている。

ガイドラインの作成には，国際的に標準化された方法に基づく，適正な評価が必要である。がん検診の有効性評価の指標は死亡率減少効果であり，発見率や生存率は適切ではない。その証拠には，無作為化比較対照試験，コホート研究，症例対照研究などにより死亡率減少を証明した直接的証拠と，Analytic Frameworkに基づき，検査精度，発見がんの病期や生存率などの種々の証拠を集積した間接的証拠がある。さらに，不利益も含めた検討が必要である。新たに作成されたがん検診ガイドラインは，両者に基づき，対策型検診と任意型検診として推奨を提示している。今後も同様の手順に基づき，がん検診ガイドラインの更新が進められる予定である。

キーワード：がん検診，有効性評価，死亡率減少

Cancer Statistics Digest

Comparison of Laryngeal Cancer Mortality in Five Countries: France, Italy, Japan, UK and USA from the WHO Mortality Database (1960–2000)

Laryngeal cancer mortality age-standardized rates (ASRs), using 1985 Japanese standard population, are shown for Japan, USA, UK, France and Italy (Fig. 1). In all of the countries, males have higher ASRs compared with females. For males, ASRs have been decreasing since 1970s in Japan and France. ASRs in the other countries have been gradually decreasing in recent years. For females in Japan, ASRs drastically decreased until 1990 and since then have been slightly decreasing. In Italy, a mild decreasing trend is observed after the middle of the 1980s. In the USA, ASRs increase until 1980. The others remained roughly flat for four decades.

Mortality trends in males are shown by age group according to year of death (Fig. 2). In Japan, the USA and the UK, decreasing trends are observed among age groups under 70 years old. In France and Italy, mortality rates are higher than in the other three countries for all age groups and there are decreasing trends after passing peaks between 1970 and 1980. Japan has the greatest difference in mortality rates between the 40–44 and 85+ age groups, while France and Italy have only a small difference between those age groups. Mortality trends in females are shown by age group according to year of death (Fig. 3). In Japan, mortality rates have been decreasing for all age groups. There is no obvious trend in the other countries.

Mortality trends in males are shown by age group according to year of birth (Fig. 4). In Japan, mortality rates decreased from the birth cohort born in 1900 onwards. In the USA, a mild decreasing trend is observed from the birth cohort born 1920. In the UK, mortality rates have been decreasing with the birth cohort born before 1920. After the birth cohort born in 1920, however, a decreasing trend is not observed. In France and Italy, mortality rates in the 40–64 age groups exhibit a peak with the birth cohort born around 1930. Mortality trends in females are shown by age group according to year of birth (Fig. 5). Decreasing trends are observed from the birth cohort born in 1900 in all of the countries except the USA. In the USA, as well as for the 40–64 age group for males in France and Italy, there is a peak in mortality rates with the birth cohort born around 1930.

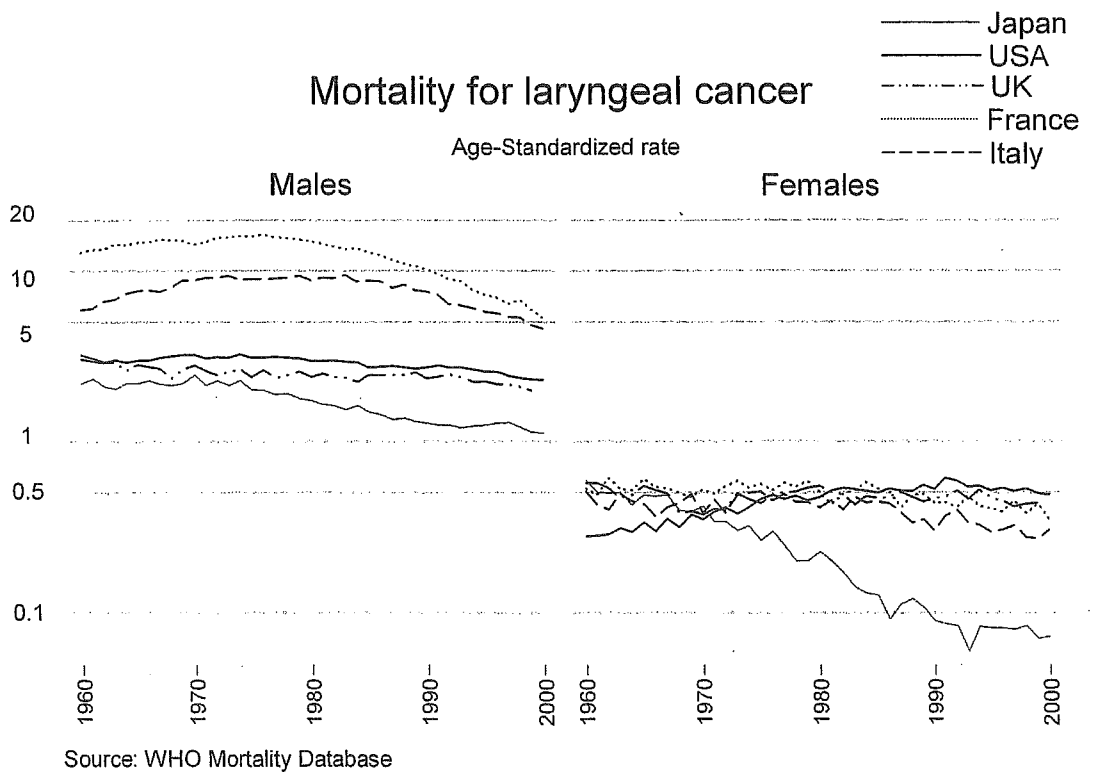

Figure 1. ASRs for laryngeal cancer for males and females: age-standardized with 1985 Japanese standard population, rates per 100 000.