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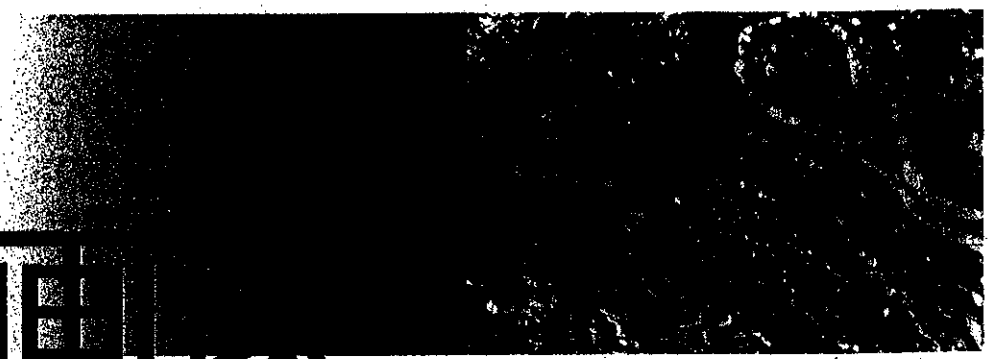
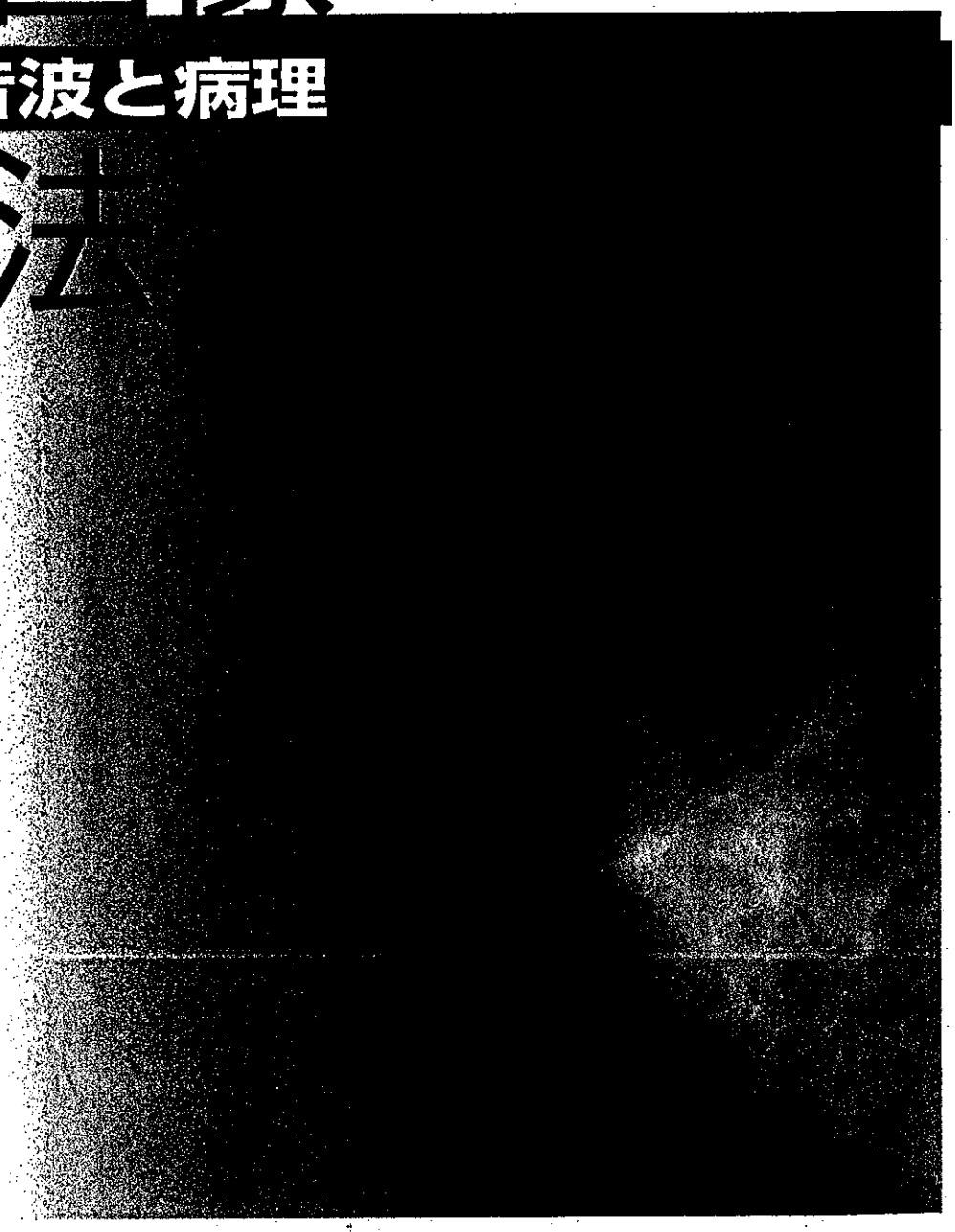


乳腺画像診断法

X線・超音波と病理



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乳腺画像
X線・超音波と病理
診断法

「乳腺診断アトラス」改訂・改題

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序

前著「乳腺診断アトラス」が出版されたのは1988年のことである。日本でも乳がんが増加してきたことから乳がん検診が始められ，また一方では乳腺専用のX線や超音波の装置が世に出始めた頃である。しかし，このような状況にもかかわらず，その当時，乳腺診断に関する邦文の適切な教科書が存在しなかった。そこで乳腺診断に携わる人たちが乳腺の画像を理解しやすいように画像を中心とした著書を出版したのである。この著書の特徴は，ほとんどの症例にX線，超音波および病理の写真が揃えられ，しかも，それぞれ最高の写真が掲載されていることである。症例ごとにX線と超音波を掲げ，それに病理組織学的な裏づけがなされている図譜は今日までなお世界に類のないものであると自負している。そして16年経た現在でもX線と超音波の写真は十分に通用するものなので，いまだにこの著書が多くの方々に利用されている。

しかしながら，X線も超音波も装置は16年前と現在とではかなり異なっている。当時の超音波検査には水浸式機械走査法装置が用いられていたが，現在では走査法が全く違う電子走査法装置が一般的になっている。X線に関しては基本的にはほとんど変わらないが，撮影装置部分で新しいX線管やステレオ撮影装置などが開発されて，撮像方式としてデジタル・ラジオグラフィが用いられるようになった。また，最近CTやMRIが乳腺の診断に導入され，普及しつつある乳がんの乳房温存手術の適応決定に際して，重要な役割を果たすようになってきている。現在これらについて記述された著書が求められているのである。そこで「乳腺診断アトラス」に掲載されている貴重な症例の写真をそのまま利用して，新たに乳腺の画像診断の著書を企画した。

従来，乳がん検診は視触診によって行われていたが，視触診だけでは見落としが多いのでX線マンモグラフィが併用されることになった。触知不能な乳がんはX線によって石灰化像が描出することで発見されるのである。しかし，乳腺が密に存在する乳房では触診やX線では病変を発見できないことがあり，その場合，超音波が必要となる。またX線検査の対象となっていない40歳以下の女性の検査には超音波は必要不可欠である。乳がんの検査にはX線と超音波は必須で，検診においても可能であれば両方を行うべきである。

乳腺の診断能を高めるには，X線と超音波の特徴を理解したうえで，それら

の画像を同時に対比し，病理組織学的変化で裏付けながら学ぶことである。この新版「乳腺画像診断法」では，総論で検査装置と検査法について記述し，各論ではすべての症例にX線，超音波，病理の写真を掲載し，診断法について解説している。この著書が乳腺検査に携わる多くの方々や乳腺診断を学ぶ方々に利用されて，乳がん検診の精度向上の役に立つことを願って止まない。

2004年6月

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Mutations of the *Epidermal Growth Factor Receptor* Gene in Lung Cancer: Biological and Clinical Implications

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ABSTRACT

Recently it has been reported that mutations in the tyrosine kinase domain of the *epidermal growth factor receptor* (*EGFR*) gene occur in a subset of patients with lung cancer showing a dramatic response to EGFR tyrosine kinase inhibitors. To gain further insights in the role of EGFR in lung carcinogenesis, we sequenced exons 18–21 of the tyrosine kinase domain using total RNA extracted from unselected 277 patients with lung cancer who underwent surgical resection and correlated the results with clinical and pathologic features. *EGFR* mutations were present in 111 patients (40%). Fifty-two were in-frame deletions around codons 746–750 in exon 19, 54 were point mutations including 49 at codon 858 in exon 21 and 4 at codon 719 in exon 18, and 5 were duplications/insertions mainly in exon 20. They were significantly more frequent in female ($P < 0.001$), adenocarcinomas ($P = 0.0013$), and in never-smokers ($P < 0.001$). Multivariate analysis suggested *EGFR* mutations were independently associated with adenocarcinoma histology ($P = 0.0012$) and smoking status ($P < 0.001$), but not with female gender ($P = 0.9917$). In adenocarcinomas, *EGFR* mutations were more frequent in well to moderately differentiated tumors ($P < 0.001$) but were independent of patient age, disease stages, or patient survival. *KRAS* and *TP53* mutations were present in 13 and 41%, respectively. *EGFR* mutations never occurred in tumors with *KRAS* mutations, whereas *EGFR* mutations were independent of *TP53* mutations. *EGFR* mutations define a distinct subset of pulmonary adenocarcinoma without *KRAS* mutations, which is not caused by tobacco carcinogens.

INTRODUCTION

Non–small-cell lung cancer (NSCLC) frequently overexpresses receptors of the erbB family including the epidermal growth factor receptor (EGFR) encoded by erbB-1 (HER1; ref. 1, 2). The EGFR is a 170 kilodaltons receptor tyrosine kinases (TK) that dimerizes and phosphorylates several tyrosine residues after binding of several specific ligands (1). These phosphorylated tyrosines serve as the binding sites for several signal transducers that initiate multiple signaling pathways resulting in cell proliferation, migration, and metastasis, evasion from apoptosis, or angiogenesis, all of which are associated with cancer phenotypes (1). Downstream pathways include ras-raf-MEK-ERK (raf-mitogen-activated protein kinase kinase-extracellular signal-regulated kinase), phosphatidylinositol-3 kinase-AKT and PAK-JNKK-JNK (p21-activated protein kinase–c-Jun NH_2 terminal kinase kinase–c-Jun NH_2 terminal kinase; ref. 1). Gefitinib is an orally administered small molecule that specifically inhibits EGFR tyrosine phosphorylation (3). Clinical trials revealed that there was a significant variability in response to gefitinib. Good clinical response has been observed most frequently in women, nonsmokers, patients with adenocarcinomas, and Japanese patients (4, 5). However, it has not been possible to predict gefitinib sensitivity by levels of EGFR overexpression as determined by immunohistochemistry (6) or immunoblotting (7). The factor(s) that determine gefitinib sensitivity has long been an enigma. It has been reported recently that activating mutations of EGFR are present in a subset of pulmonary adenocarcinomas and that tumors with *EGFR* mutations are highly sensitive to gefitinib (8, 9). Furthermore, the incidence of *EGFR* mutations is higher in Japanese than in Caucasian patients (8). In this study, we searched for *EGFR* mutations in a large cohort of unselected Japanese NSCLC to correlate them with clinical and pathologic features including *KRAS* or *TP53* mutations.

MATERIALS AND METHODS

Patients. Primary tumor samples were obtained from 277 unselected patients with lung cancer who underwent potentially curative pulmonary resection at the Department of Thoracic Surgery, Aichi Cancer Center Hospital from May, 2000 through November, 2000 and from January, 2001 through December, 2002, after obtaining appropriate approval from the institutional review and patients' written informed consent. These cases corresponded to 82% of all consecutive cases. Inclusion of the cases into this study was dependent on availability of frozen tumor material. About 20 cases were excluded because tumor cells were too few to sufficiently extract tumor RNA because of inflammation and/or necrosis. There were 159 males and 118 females with an age at diagnosis ranging from 26 to 89 (median 64) years. One hundred fifty-nine patients had stage I disease, 39 had stage II, 74 had stage III and 5 had stage IV diseases. There were 224 adenocarcinomas, 35 squamous cell carcinomas, 9 large cell carcinomas, 5 adenosquamous carcinomas, 3 small cell carcinomas, and 1 carcinoid. There were 115 never-smokers and 162 ever-smokers including current and former smokers. Smoking history was obtained by interviewing each patient at admission or first outpatient visit.

Molecular Analysis of Lung Cancer Specimens. Tumor samples were obtained at the time of surgery, rapidly frozen in liquid nitrogen, and stored at −80°C. Frozen tissue of the tumor specimens were grossly dissected to enrich as much tumor cells as possible by a surgical pathologist (Y. Y.). We isolated total RNA using the RNAeasy kit (Qiagen, Valencia, CA).

The first four exons (exons 18–21) of the seven exons (exons 18–24) that code for TK domain of the *EGFR* gene that includes all of the mutations reported thus far (8, 9) were amplified with primers F1 (5′-AGCTTGTGGAGCCTCTTACACC-3′) and R1 (5′-TAAAATTGATTCCAATGCCATCC-3′), in a one-step reverse transcription-PCR setup with Qiagen OneStep reverse transcription-PCR kit (Qiagen, Valencia, CA). The cDNA sequence of *EGFR* gene was obtained from GenBank (accession number NM005228). Reverse transcription-PCR conditions were available after request. Reverse transcription-PCR products were diluted and cycle-sequenced with the Big Dye Terminator v3.1/1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Sequencing reactions were electrophoresed on an ABI PRISM 3100 (Applied Biosystems). Both the forward and reverse sequences obtained were analyzed by BLAST and chromatograms by manual review.

***KRAS* and *TP53* Gene Analysis.** We had previously examined the same cohort for *KRAS* mutations and *TP53* mutations (10, 11). Briefly, *TP53* gene (exon 4 through 10) and *KRAS* gene (exons 1 and 2) were amplified and directly sequenced with ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Statistical Analysis. For comparisons of proportions, the χ^2 test or Fisher's exact test were used. The Kaplan-Meier method was used to estimate the probability of survival as a function of time, and survival differences were

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Note: T. Kosaka and Y. Yatabe contributed equally to the present study.

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analyzed by the log-rank test. The two-sided significance level was set at $P < 0.05$. To identify which independent factors jointly had a significant influence on the incidence of EGFR mutations, the logistic regression modeling technique was used. We did all analyses using a StatView (version 5, SAS Institute Inc., Cary, NC) software on a Macintosh computer.

RESULTS

***EGFR* Mutations in Unselected Lung Cancer Specimens.** Of 277 unselected patients who underwent surgical resection of their tumors, we found that 111 patients (40%) had mutations in exons 18–21 of the *EGFR* gene. There were 52 deletion mutations, 54 point mutations, and 5 duplication/insertion mutations. In 14 tumors, corresponding cDNA from normal lung tissue far from the tumors was also sequenced, which confirmed that all these mutations were somatic. Details of the resulting changes in EGFR protein as a consequence of these mutations are illustrated in Fig. 1.

All of the 52 deletion mutations occurred around codons 746–750 in exon 19. About half (25 of 52) of deletion mutations were simple deletions of five amino acid residues ELREA from codon 746 to 750. However, 22 deletions were coupled with point mutations or insertions, yielding various changes in amino acid sequences as shown in Fig. 1. It is noted that, in all cases, such alterations were in-frame. Forty-six of the 54 point mutations were from a T to a G transversion at the second nucleotide of codon 858 in exon 21 resulting in substitution of leucine with arginine residue. Four of the point mutations occurred at codon 719 in exon 18. We noted that one tumor with a mutation at codon 719 and three tumors with mutations at codon 858 had another mutation occurring at codons 709, 768, 776, and 790, respectively. For rare mutations (all 5 insertions, E709H, T790M, S768I, R776C, V769L), we resequenced and confirmed that these mutations were actually present. In summary, 52 of the 111 (47%) *EGFR* mutations were deletions around codons 746–750 and 49 (44%) were L858R, altogether accounting for 91% of all of the *EGFR* mutations found. The four major classes of mutations (*i.e.*, deletions, L858R, mutations at codon 719, duplications/insertions) never occurred simultaneously. Furthermore, it is of note that only mutant sequences were present in chromatograms in 19 of 52 deletions, 13 of 46 in L858R, and 1 of 4 codon 719 mutations.

Relationship between *EGFR* Mutations and Clinical-Pathologic Features. *EGFR* mutations were significantly more frequent in females (59%) than males (26%; $P < 0.001$), in never-smokers (66%) than ever-smokers (22%; $P < 0.001$), and in patients with adenocarcinomas (49%) than in those with nonadenocarcinomas (2%; $P < 0.001$). There was only one patient with an *EGFR* mutation of 53 nonadenocarcinoma patients. This patient was a 61-year-old male with adenosquamous carcinoma. Because female patients tended to be never-smokers and were likely to have adenocarcinoma, we did logistic regression analysis to determine which of these three variables independently contributed to the *EGFR* mutations. The result suggested that smoking status and adenocarcinoma histology independently affected *EGFR* mutations whereas female gender did not (smoking status, odds ratio 3.949, $P < 0.001$; histologic type, odds ratio 27.486, $P = 0.0013$; gender odds ratio 0.996, $P = 0.9917$).

Further Analysis of Patients with Adenocarcinoma. *EGFR* mutations were found almost exclusively in adenocarcinomas with only one exception; hence, we did more detailed analysis limited to this subset of patients (Table 1). *EGFR* mutations were also significantly frequent in female, nonsmoking patients. When we divided ever-smokers into 3 categories depending on smoke exposure, there was a trend that the higher the exposure, the lower the incidence of *EGFR* mutations. *EGFR* mutations were significantly more frequent in well to moderately differentiated adenocarcinomas (58%) than in poorly differentiated adenocarcinomas (30%; $P < 0.001$). There were five bronchioloalveolar cell carcinomas (BAC) in our cohort, of which three harbored *EGFR* mutations (60%), according to the World Health Organization classification of lung cancers (which states that BAC is a true noninvasive cancer without stromal or pleural invasion; ref. 12). It seemed that *EGFR* mutations were associated neither with age of the patients nor with stage of diseases. There was no difference in incidence of *EGFR* mutations between both sexes in patients of age 50 (average age of menopause in Japan) or younger, although the number of patients of this age group was small (2 of 7 males, 2 of 7 females).

Our preliminary study indicated that patients with *EGFR* mutations survived for a longer period after gefitinib treatment than those without *EGFR* mutations.[5] However, *EGFR* mutations also might have prognostic impact on patients with pulmonary adenocarcinoma, even when the patients were not exposed to gefitinib because EGFR

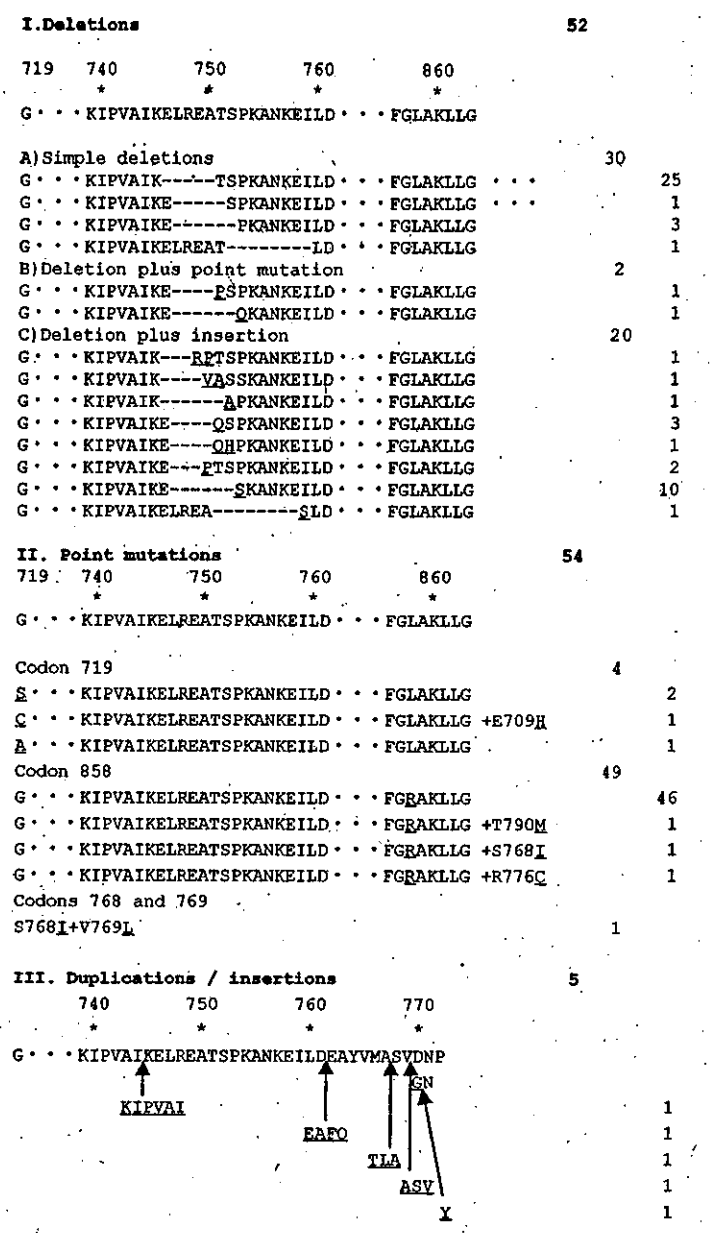

Fig. 1. Analysis of 111 *EGFR* mutations in the TK domain of the *EGFR* gene found in unselected cases with lung cancer.

[5] T. Mitsudomi, T. Kosaka, H. Endoh, Y. Horio, T. Hida, S. Mori, S. Hatooka, M. Shinoda, T. Takahashi, Y. Yatabe, submitted for publication.

Table 1 *Relationship between EGFR mutations and clinical and pathologic features in a subset of patients with adenocarcinoma*

Variables	Category	EGFR			P
		Mutation (%)		Wild-type	
N		110	(49)	114	
Gender	Male	40	(36)	71	<0.001
	Female	70	(62)	43	
Age	≤64	51	(46)	60	0.3481
	> 64	59	(52)	54	
Smoking status	Never-smoker (pack years = 0)	76	(68)	36	<0.001
	Ever-smoker	34	(31)	78	
	Pack years <20	11	(55)	9	
	20–50	15	(27)	40	
	>20	8	(22)	29	
Differentiation	Well to moderately differentiated	89	(58)	65	<0.001
	Poorly differentiated	21	(30)	49	
Stage	IA and IB	69	(50)	70	0.8383
	IIA through IV	41	(48)	44	
Survival	3-year survival rate	86%		91%	0.9933
KRAS mutation	Mutated	0	(0)	26	<0.001
	Wild-type	97	(57)	73	
TP53 mutation	Mutated	37	(47)	42	0.4634
	Wild-type	59	(52)	54	

There were five BACs in our cohort, of which three harbored *EGFR* mutations.

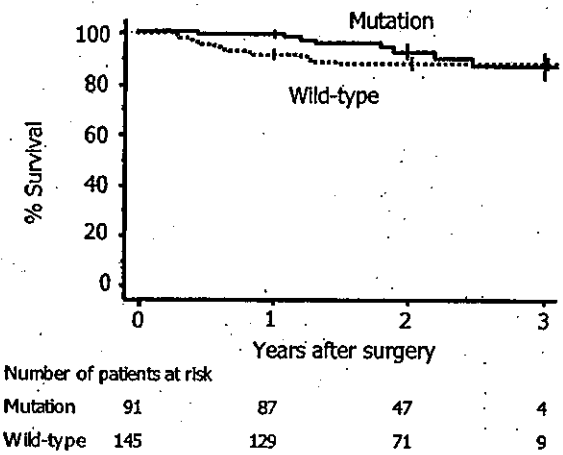

Fig. 2. Effect of *EGFR* mutations on survival of patients with adenocarcinoma calculated from the day of surgery. Patients later treated with gefitinib and those whose surgery was done for recurrent or second primary cancers were excluded.

mutations defined subsets of pulmonary adenocarcinoma with distinct features as described. Therefore, we did survival analysis in patients excluding those who were treated with gefitinib when they had recurrent diseases. The Kaplan-Meier curve (Fig. 2) indicated that EGFR mutations did not affect prognosis of the patients ($P = 0.9933$), although the follow up period was relatively short (median follow up, 788 days)

***KRAS* and *TP53* Gene Mutational Analysis.** Of 224 patients with adenocarcinoma, *KRAS* and *TP53* data were available for 196 and 192 patients, respectively. *KRAS* mutations were present in 26 of 196 patients (13%; 22 at codon 12, 1 at codon 13, and 3 at codon 61). *TP53* mutations were present in 79 of 192 (41%). *KRAS* and *TP53* mutations were significantly more frequent in ever-smokers, respectively [20% *versus* 6% for *KRAS* ($P = 0.0054$) and 54% *versus* 30% for *TP53* ($P < 0.001$)]. Interestingly, *EGFR* mutations were never found in tumors with *KRAS* mutations, showing a mutually exclusive relationship. By contrast, *EGFR* mutations and *TP53* mutations seemed to occur independently. Figure 3 shows the relationship among the three mutations by a Venn diagram in 192 patients in whom information about the status of these three genes was available.

TP53 mutations seemed more widely distributed in tumors without *EGFR* mutations (Fig. 4). Of seven mutations either at codon 157, 248, or 273 in which strong and selective adduct formation of benzo(*a*)pyrene diol epoxide, one of the major tobacco carcinogens, occurs (13), six were in tumors without *EGFR* mutations (Fig. 3). Furthermore, of 16 mutations caused by a G to a T transversions characteristic of mutations caused by aromatic polycyclic hydrocarbons (14), 15 were in tumors without *EGFR* mutations (Fig. 3).

DISCUSSION

Adenocarcinoma is the most predominant histologic subtype, and its incidence is increasing in Japan. Registration of resected lung cancer in Niigata prefecture, Japan, revealed that the incidence of adenocarcinoma is 71% of 1211 patients operated on from 2001 to 2002 (15). In our institution, adenocarcinoma accounted for 54% of 975 patients who were operated on from 1965 through 1995, 69% of 522 from 1996 through 2000, and 76% of 407 from 2001 through 2003. Considerable evidence indicates that the EGFR pathway also plays an important role in both the pathogenesis and the progression of lung cancer (1).

We found that 40% of 277 unselected patients with lung cancer carried mutations in the TK domain of the *EGFR* gene. More than 90% of the mutations were either deletions around codons 746–750 in

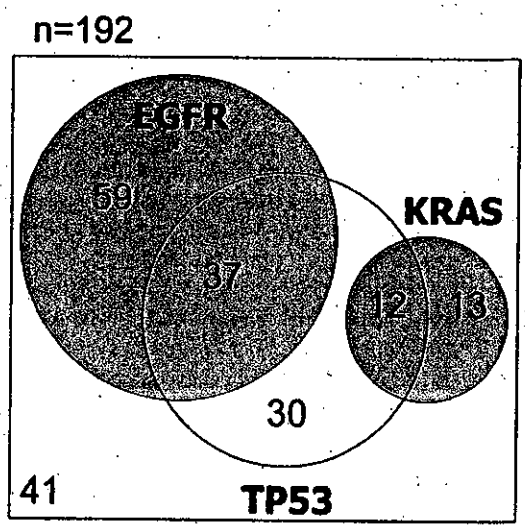

Fig. 3. The Venn diagram illustrating relationship among *EGFR* mutations, *KRAS* mutations and *TP53* mutations in patients with adenocarcinoma ($n = 192$). Diameters of each circle are roughly proportional to the number of mutations.

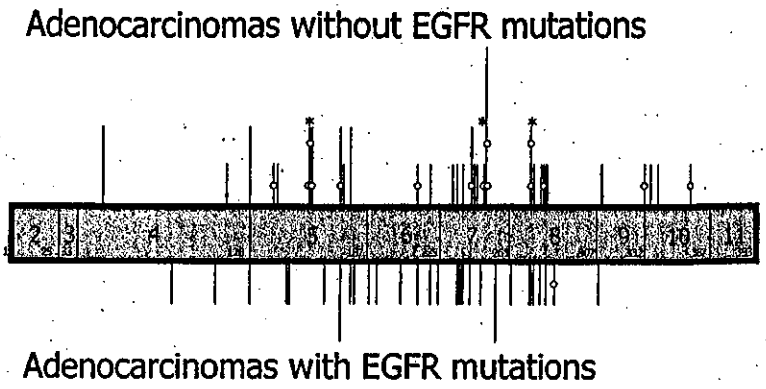

Fig. 4. Distribution of *TP53* gene mutations in adenocarcinomas without *EGFR* mutations (n = 42) or with *EGFR* mutations (n = 32). Numbers below show codons of exon boundaries. *Asterisks* show codons 157, 248, and 273, where strong and selective benzo(*a*)pyrene diol epoxide adduct formation is reported to occur (13). *White circles* indicate where *TP53* mutations were caused by a G to a T transversion.

exon 19 or L858R in exon 21, which all flank the ATP-binding pocket that is important for TK activity (8, 9). We also noted that in about 30% of the cases with *EGFR* mutations, only bands derived from mutant allele were detected on chromatogram. This is somewhat puzzling considering the heterozygous nature of the *EGFR* mutations reported thus far (8, 9) and the presence of stromal cells in resected tumor specimens. This finding may suggest that loss of wild-type alleles or amplification of mutant alleles accompanied with mutations in these cases, as indicated by Minna *et al.* (16).

EGFR mutations were almost exclusively present in adenocarcinoma. Mutations were more prevalent in females and nonsmokers, confirming and extending the results of previous reports (8, 9). It is noteworthy that these characteristics and Japanese ethnicity are all predictors of gefitinib sensitivity at least by univariate analysis (4, 5). Multivariate analysis suggested that nonsmoking status and adenocarcinoma histology independently contributed to *EGFR* mutations but female gender did not. The fact that premenopausal women did not show higher incidence of *EGFR* mutations further suggested that apparent difference between female and male was caused by a difference in lifestyle including smoking habit rather than involvement of sexual environment.

Previously described genetic alterations in lung cancer are almost always more frequent in smokers than nonsmokers. For example, mutations of the *TP53* gene (17), *KRAS* genes (18), or deletion of the short arm of chromosome 3 (19) are known to be more frequent in smokers, as was the case in the present study for the first two. A plausible explanation for the reason why *EGFR* mutations are associated with nonsmoking status are not possible at this time, but it is natural to assume that *EGFR* mutations are caused by carcinogen(s) other than those contained in tobacco smoke. In Taiwan, human papilloma virus type, 16 of 18 infections (20) or cooking oil fume (21) have been investigated as a cause of lung cancer occurring in nonsmoking women. These observations might be relevant with preferential *EGFR* mutations in nonsmoking women. Nevertheless, *EGFR* mutations should provide a clue for pathogenesis of adenocarcinoma occurring in nonsmokers and should ultimately lead to discovery of effective prevention.

We were able to confirm higher incidence of *EGFR* mutations in Japanese patients. Lynch *et al.* found *EGFR* mutation in 2 of 25 unselected United States patients (9), and Paez *et al.* (8) did so in 1 of 61 United States patients and 15 of 58 Japanese patients. The reason for this marked difference between Japanese and United States patients is not very clear. However, difference in incidence of nonsmoking patients between Japanese and American female patients with lung cancer may partly account for this. In our cohort, 83% of female patients and 10% of male patients were never-smokers. This trend is common in Japan. For example, Toyooka *et al.* (22) and Minami *et al.* (23) reported that the proportion of never-smoking women in lung cancer patients is 96% and 75%, respectively. This makes quite a contrast with the fact that only 15% of 706 United States female and 6% of 1,347 male patients with lung cancer are never-smokers (24).

We found that *EGFR* mutations and *KRAS* mutations known to play an important role in pathogenesis of adenocarcinoma of the lung (25) were strictly mutually exclusive, reminding us of a similar exclusionary relationship between retinoblastoma and p16 inactivation in lung cancer (26). This finding may be explained by the fact that the KRAS-mitogen-activated protein kinase pathway is one of the downstream signaling pathways of EGFR (1). Because it has been shown that L858R and delL747-P753ins S are activating mutations that result in markedly increased phosphorylation of EGFR when EGF was added (8, 9), tumors with *KRAS* mutations that already have activated further downstream effectors do not need to have *EGFR* mutations. The high incidence of *EGFR* mutations in lung adenocarcinomas may explain why *KRAS* mutations are lower in Japanese than in Caucasian patients. In the present study, *KRAS* mutations were found in 13% of adenocarcinomas, whereas they were present in 33% of Dutch cases (25). This may be also at least partially attributable to the difference in smoking status, because *KRAS* mutations were more frequent in smokers as reported previously (18). In contrast, the incidence of *TP53* mutations was not associated with *EGFR* mutations, although *TP53* mutations also occurred more frequently in smokers (17). However, *TP53* mutations in tumors without *EGFR* mutations showed characteristics of mutations caused by tobacco carcinogens in terms of sites or base substitution patterns (13, 14).

We also noted that well to moderately differentiated adenocarcinomas had a significantly higher incidence of *EGFR* mutations than poorly differentiated ones. This observation might be relevant to the fact that adenocarcinomas showing BAC feature show higher sensitivity to gefitinib (27). However, when we used the strict criteria as stated by the World Health Organization Classification of lung tumors (12), our cohort included only five BAC, of which three had EGFR mutations. Unfortunately, these strict criteria are not applied by many pathologists, leading to considerable confusion between BAC and adenocarcinoma with BAC features in the literature. Alternatively, we proposed terminal respiratory unit type adenocarcinoma that is characterized by morphological resemblance to type II pneumocytes, Clara cells, and/or bronchioles as well as expression of thyroid transcription factor-1 and surfactant proprotein B (refs. 28, 29). In the World Health Organization classification, most nonmucinous bronchioloalveolar, mixed bronchioloalveolar and acinar subtypes, and some papillary subtypes belong to the terminal respiratory unit type adenocarcinoma (28, 29). We found that most adenocarcinoma with EGFR mutations were categorized into terminal respiratory unit type adenocarcinoma.[6]

[6] Y. Yatabe, T. Kosaka, T. Takahashi, T. Mitsadomi, submitted for publication.

EGFR mutations were not associated with stage of disease, suggesting that EGFR mutations occurs relatively early in clinical course and are associated with pathogenesis of adenocarcinoma rather than progression.

In conclusion, we found a high incidence of EGFR mutations in Japanese patients with pulmonary adenocarcinoma, especially in those who never smoked. EGFR mutations were never present in tumors with KRAS mutations, indicating possibilities of genotype-oriented approach for pulmonary adenocarcinoma.

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濱島 ちさと

近年，諸外国ではがん検診の有効性を評価し，公共政策に活用する動きが見られる．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．その評価方法は，USPSTF(『US Preventive Services Task Force』第2版)を参考にしている．そのUSPSTFにおいて，ガイドラインの更新にあたり，2001年から新しい評価方法が取り入れられた[1]．

USPSTFの新しい評価方法

ガイドラインの推奨の基準は，研究方法とその根拠を示すことで，その推奨のレベルが決定されてきた．USPSTFの第1・2版も基本的にはこの方法を踏襲したものであったが，第3版の改正では，推奨の基準と研究の評価方法が修正された．

変更点の第1は，推奨基準である．推奨基準は第2版でも5段階方式が採用されていた．このうち，A，Bは推奨，Cは保留，D，Eが非推奨であった．第3版では，この形式を修正し，表1の5段階を採用している．第2版と第3版の評価を比べる場合には，この推奨形式の変更に留意する必要がある．

表1 USPSTFの推奨基準(Harris RP, 2001より)

推奨	表現
A	USPSTFは，臨床家が適格な患者に対して日常的に当該サービスを提供することを強く勧告する．(USPSTFは，当該サービスが重要な健康指標を改善することを示す優良な証拠があると判断し，利益が不利益を大きく上回ると結論する)
B	USPSTFは，臨床家が適格な患者に対して日常的に当該サービスを提供することを勧告する．(USPSTFは，当該サービスが重要な健康指標を改善することを示す少なくとも相応の証拠があると判断し，利益が不利益を上回ると結論する)
C	USPSTFは，当該サービスを日常的に提供することについて，勧めることも反対することもしない．(USPSTFは，当該サービスが重要な健康指標を改善することを示す少なくとも相応の証拠があると判断するが，一般的な勧告を正当化するには利益と不利益のバランスが近接しすぎていると結論する)
D	USPSTFは，当該サービスを日常的に無症状の患者に対して提供することに反対する．(USPSTFは，当該サービスが効果がない，あるいは，不利益が利益を上回るとする少なくとも相応の証拠があると判断する)
I	USPSTFは，当該サービスを日常的に提供することについて，勧めるまたは反対する勧告を出すための証拠が不十分であると結論する．(当該サービスに効果があるとする証拠がないか，質が悪いか，あるいは，一致した結果が得られていないため，利益と不利益のバランスを判断できない)

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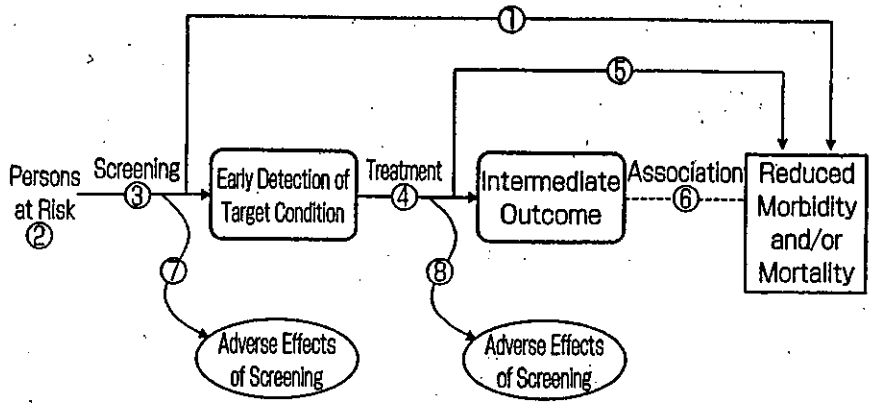

図　USPSTFにおける検診のAnalytic Framework

[Harris RP, et al: Current methods of the U. S. Preventive Services Task Force; A review of the process. Am J Prev Med 20 (suppl 3): 21-35, 2001 より]

表2　USPSTFの推奨グリッド

証拠の質	利益と不利益の差(Net Benefit)			
	大きい	中等度	小さい	ゼロ/マイナス
Good	A	B	C	D
Fair	B	B	C	D
Poor	I			

第2は研究方法ばかりではなく，研究の質を吟味することが追加された．研究方法にさらに質の検討を加え，Good・Fair・Poorの3段階で評価する．第2版までの評価方法においても，研究の質はある程度考慮されてはいたが，評価の主体はどのような研究方法により根拠が示されているかが，最も重要な点であった．この結果，無作為化比較対照試験(Randomized Controlled Trial: RCT)により評価された医療サービスの評価が最も高く，観察研究は評価が下がってしまう．RCTであれば即信頼性が裏付けられるかというと必ずしもそうではなく，一定の質が求められる．RCT一辺倒の評価に疑問を投げかけたのが，コクランの乳がん検診の評価である．この論争を契機に，研究方法だけではなく，研究のデザインや利益・不利益を重視した評価が再認識された．USPSTFにおける乳がん検診の評価も，これまで最高の推奨でAであったものから，1ランク落としたBに変更された．

第3は，評価対象となる予防対策や検診について，利益と不利益を勘案した評価方法が追加された．あらゆる医療サービスには，利益・不利益があることが指摘されてきたが，その評価をガイドラインにどのように組み込むかは，必ずしも統一見解があるわけではない．しかし近年，臨床ガイドラインの作成過程においては，利益・不利益を何らかの形で評価することが求められる．

USPSTFでは，推奨グリッド(表2)に基づき，研究の質と利益と不利益の差(Net Benefit)を用いて評価しようというものである．ここでいう利益とは，医療サービスが質の高い研究により，その根拠の裏づけを得ていることである．がん検診であれば，死亡率減少効果が示されていることである．利益の評価のためには信頼性に高い研究方法と質によりその判定が異なる．一方，不利益は，医療サービスがもたらすマイナスの側面で，検査の見逃しや，過剰診断，検査や治療の合併症である．これらは，従来から指摘されていた問題であった．ガイドラインの中にどのように組み込むかが明確ではなかったが，今回の推奨には「利益が不利益をどの程度上回るか」を判定することにより，推奨の段階に反映する仕組みが作られている．

第4は，直接的な根拠だけでなく，間接的根拠についても評価を行うためのAnalytic Frameworkが導入された(図)．第2版にも類似のCausal Pathwaysが存在していたが，第3版においては，直接的な証拠がない場合でも，Analytic Frameworkの各段階におけるKey Ques-

tionに対応する研究を積み重ねることで，検診や予防対策を評価しようというものである．

がん検診の新たな評価

USPSTF第3版の更新方法の変更が公表されて以来，第2版の評価に新たな研究を加え，評価の更新が行われつつある．2004年8月までのがん検診の更新は表3のとおりである．新たな推奨段階では，CとIが類似しているが，実際にCと判定される可能性は少なく，科学的根拠が不十分であるとするIの評価が多い．

USPSTFにおける大腸がん検診評価

大腸がん検診の評価を見ると，個々の検診方法の評価ではなく，大腸がん検診として推奨Aの判定を受けている[2]．ただしその方法には，便潜血検査，S状結腸鏡，便潜血検査とS状結腸鏡の併用法，全大腸内視鏡検査，注腸造影のすべてをまとめた上での評価が行われており，個別の検診方法の評価は明確ではない．しかし，3つにRCTにより有効性評価が確立している便潜血検査のみである[3]．

化学法による便潜血検査による大腸がん検診の死亡率減少効果は，3件の無作為化比較対照試験によりその直接的な根拠が証明されている．米国Minnesota[4]では，50～80歳の男女を対象に，隔年受診群(15,587例)，逐年受診群(15,570例)，対照群(15,394例)の3群について，18年間にわたる追跡を行った．対照群に比し，隔年受診群で21%(RR=0.79；95%CI 0.62～0.97)，逐年検診で33%(RR=0.67；95%CI 0.51～0.83)の大腸がん死亡抑制効果が認められた．英国Nottingham[5]では，45～74歳を対象とし，逐年受診群(76,224例)，対照群(76,079例)について11年間にわたる追跡を行い，隔年受診群で13%の大腸がん死亡抑制効果を認めた(RR=0.87；95%CI 0.78～0.97)．デンマークFunen[6]では，45～75歳を対象とし，逐年受診群(30,967例)，対照群(30,966例)について13年間にわたる追跡を行い，隔年受診群で18%(RR=0.82；95%CI 0.69～0.97)の大腸がん死亡抑制効果を認めた．スウェーデンの無作為化比較対照試験の中間報告を加えた4件の無作為化比較対照試験のメタ・アナリシス[7]では，16%の死亡率減少効果(RR=0.84；95%CI 0.77～0.93)が認められている．ただし，USPSTFによる便潜血検査の評価は化学法を対象としたものであり，わが国に普及している免疫法は対象外となっている．

この他，S状結腸鏡，便潜血検査とS状結腸鏡の併用法，全大腸内視鏡については，症例対照研究やコホート研究による科学的根拠は示されてはいるものの，それは便潜血検査に比し薄弱なものである．また，注腸造影についても，全大腸内視鏡の代替案として評価されている．

表3　USPSTFの評価の更新

がん検診	検診方法	更新年	推奨	前回の評価
膀胱がん	尿検査・尿細胞診・BTA・NMP22	2004	D	D(1996)
肺がん	胸部CT・胸部X線・喀痰細胞診	2004	I	D(1996)
すい臓がん	診察・US・腫瘍マーカー	2004	D	D(1996)
精巣がん	診察・US・腫瘍マーカー	2004	D	C(1996)
口腔がん	診察・自己触診	2003	I	C(1996)
子宮頸がん(21～64歳)	細胞診	2003	A	A(1996)
子宮頸がん(65歳以上)	細胞診	2003	D	C(1996)
子宮頸がん(子宮全摘後)	細胞診	2003	D	
子宮頸がん	HPV	2003	I	C(1996)
乳がん	マンモグラフィ	2002	B	50-69歳A(1996)，他の年齢C(1996)
乳がん	視触診	2002	I	C(1996)
乳がん	自己検診	2002	I	C(1996)
前立腺がん	PSA・直腸指診	2002	I	D(1996)
大腸がん	便潜血・S状結腸鏡・全大腸内視鏡・注腸造影	2002	A	便潜血・S状結腸鏡B(1996)，全大腸内視鏡・注腸造影・直腸指診C(1996)
皮膚がん	診察	2001	I	C(1996)
卵巣がん	CA-125・US	2001	I	D(1996)

まとめ

以上，USPSTFにおける新たな評価方法を概説するとともに，近年更新された推奨を提示した．新たに更新された評価の問題点として，大腸がん検診の評価の例を示した．ガイドラインの推奨方法，作成基準について，国際的な議論が活発化している．こうした動向を踏まえ，現在，わが国においてもがん検診の有効性評価の更新作業が進められている．

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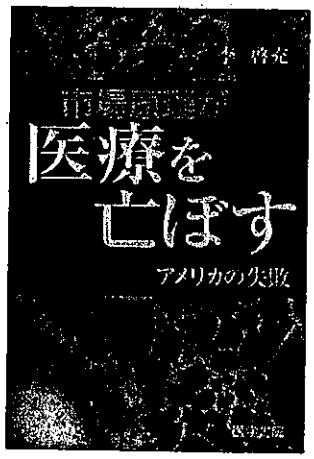
市場原理が医療を亡ぼす —アメリカの失敗



「市場原理が医療を亡ぼす—アメリカの失敗」李啓充(著)，医学書院，定価2,100円(本体2,000円＋税5%)，2004年

頻発する株式会社病院の「犯罪」，財力に基づく凄惨な医療差別……，医療に市場原理が導入された結果，米国医療はどうゆがんだか!? 優れた医事評論で知られる著者が，米国の事例を紹介しつつ，経済界主導で進む日本の医療のあり方に警鐘を鳴らす．「混合診療解禁」，「医療機関経営への株式会社の参入容認」など，医療における「ビジネス・チャンスの創出」を目論む勢力が主導する改革議論に正面から斬り込む．

これを読まずして医療改革は語れない!!

〈目次〉

Egg consumption, serum cholesterol, and cause-specific and all-cause mortality: the National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged, 1980 (NIPPON DATA80)[1–3]

Yasuyuki Nakamura, Tomonori Okamura, Shinji Tamaki, Takashi Kadowaki, Takehito Hayakawa, Yoshikuni Kita, Akira Okayama, and Hirotsugu Ueshima for the NIPPON DATA80 Research Group

ABSTRACT

Background: Because egg yolk has a high cholesterol concentration, limited egg consumption is often suggested to help prevent ischemic heart disease (IHD).

Objective: We epidemiologically examined the validity of this recommendation.

Design: We analyzed the relations of egg consumption to serum cholesterol and cause-specific and all-cause mortality by using the NIPPON DATA80 (National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980) database. At the baseline examination in 1980, a nutritional survey was performed by using the food-frequency method in Japanese subjects aged ≥30 y. We followed 5186 women and 4077 men for 14 y.

Results: The subjects were categorized into 5 egg consumption groups on the basis of their responses to a questionnaire (≥2/d, 1/d, 1/2 d, 1–2/wk, and seldom). There were 69, 1396, 1667, 1742, and 315 women in each of the 5 groups, respectively. Age-adjusted total cholesterol (5.21, 5.04, 4.95, 4.91, and 4.92 mmol/L in the 5 egg consumption categories, respectively) was related to egg consumption ($P < 0.0001$, analysis of covariance). In women, unadjusted IHD mortality and all-cause mortality differed significantly between the groups [IHD mortality: 1.1, 0.5, 0.4, 0.5, and 2.0 per 1000 person-years, respectively ($P = 0.008$, chi-square test); all-cause mortality: 14.8, 8.0, 7.5, 7.5, and 14.5 per 1000 person-years, respectively ($P < 0.0001$, chi-square test)]. In men, egg consumption was not related to age-adjusted total cholesterol. Cox analysis found that, in women, all-cause mortality in the 1–2-eggs/wk group was significantly lower than that in the 1-egg/d group, whereas no such relations were noted in men.

Conclusion: Limiting egg consumption may have some health benefits, at least in women in geographic areas where egg consumption makes a relatively large contribution to total dietary cholesterol intake. *Am J Clin Nutr* 2004;80:58–63.

KEY WORDS Eggs; total cholesterol; ischemic heart disease; National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980; NIPPON DATA80; Keys equation

INTRODUCTION

Because egg yolk has a relatively high cholesterol concentration, limited egg consumption is often recommended to reduce serum cholesterol concentrations and to help prevent ischemic heart disease (IHD) (1). Although several metabolic ward studies showed that dietary cholesterol is a major determinant of serum cholesterol concentrations (2, 3), other studies failed to show changes in serum total cholesterol concentration when eggs were added to diets that already contained moderate amounts of cholesterol (4–7). There have been few epidemiologic studies in free-living populations that explored the relation of egg consumption to serum cholesterol and IHD (8–12). A Framingham Study of 912 subjects concluded that egg consumption was not related to serum cholesterol or IHD (11). A study by Hu et al (12) of 117 933 subjects in the United States also showed no relation between consumption of ≤1 egg/d and the risk of IHD or stroke. However, in geographic areas where egg consumption makes a greater contribution to total dietary cholesterol intake than in the United States, the results may be different (13–15). Accordingly, we analyzed the relations of egg consumption to serum cholesterol concentrations and cause-specific and all-cause mortality by using the NIPPON DATA80 (National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980) database, which includes >10 000 subjects in Japan who were followed for 14 y (16–18).

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SUBJECTS AND METHODS

Subjects

The subjects in this cohort were participants in the 1980 National Survey on Circulatory Disorders (19). A total of 10 546 community-based subjects aged ≥30 y in 300 randomly selected health districts throughout Japan participated in the survey, which consisted of a medical history, physical examinations, blood tests, and a self-administered questionnaire on lifestyle, which included an essential nutritional survey performed by the food-frequency method. The cohort was followed until 1994 (NIPPON DATA80) (16–18). The overall population aged ≥30 y in the 300 participating health districts was 13 771. Therefore, the participation rate of the survey was 76.6% before exclusion for the reasons mentioned below. To clarify the cause of death, we used the National Vital Statistics. In accordance with Japan's Family Registration Law, all death certificates issued by physicians were forwarded to the Ministry of Health and Welfare via the public health centers in the district of residency. The underlying causes of death were coded according to the 9th revision of the *International Classification of Diseases* for the National Vital Statistics. We confirmed death in each health district by computer matching of data from the National Vital Statistics, with district, sex, and dates of birth and death as key codes. Of 10 546 subjects, a total of 1283 were excluded for the following reasons: past history of coronary artery disease or stroke ($n = 166$); some missing information on the baseline survey ($n = 247$); and lost to follow-up ($n = 870$). We analyzed the remaining 9263 subjects (5186 men and 4077 men). There was no significant difference in sex-specific mean total cholesterol concentration between the subjects who were lost to follow-up and those who were censored (194 compared with 192 mg/dL, respectively, in the women; 191 compared with 188 mg/dL, respectively, in the men). Therefore, the potential bias regarding the 870 subjects lost to follow-up was thought to be negligible. Permission to use the National Vital Statistics was obtained from the Management and Coordination Agency, Government of Japan. Ethical approval for this study was obtained from the Institutional Review Board of Shiga University of Medical Science (No. 12-18, 2000).

Biochemical and baseline examinations

The baseline surveys were conducted by public health centers. Baseline blood pressures were measured by trained observers using a standard mercury sphygmomanometer on the subjects' right arm while the subjects were seated and after they had rested for ≥5 min. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, use of antihypertensive agents, or any combination of these. Height in stocking feet and weight in light clothing were measured. Body mass index was calculated as weight (in kg) divided by the square of height (in m).

A lifestyle survey was carried out by using a self-administered questionnaire, which included questions about the average consumption of 31 food items. Egg consumption was queried on the basis of 5 categories: ≥2/d, ≈1/d, ≈1/2 d, ≈1–2/wk, and seldom. Public health nurses rechecked information with the subjects regarding consumption of eggs and other foods, smoking status, drinking habits, and present and past medical histories.

Nonfasting blood samples were drawn, centrifuged for 15 min at 1500 × *g* and room temperature within 60 min of collection, and then stored at −70 °C until analyses. Total cholesterol was analyzed in a sequential autoanalyzer (SMA12; Technicon, Tarrytown, NY) by using the Lieberman-Burchard direct method for total cholesterol at a single laboratory (Osaka Medical Center for Health Science and Promotion, Osaka, Japan). This laboratory is a member of the Cholesterol Reference Method Laboratory Network (20), and the precision and accuracy of measurement of serum cholesterol were certified in the Lipid Standardization Program administered by the Centers for Disease Control and Prevention, Atlanta. Serum glucose concentrations were measured by using the cupric-neocuproline method (21). Diabetes was defined as a serum glucose concentration ≥ 200 mg/dL, a past history of diabetes, or both. Serum creatinine was analyzed in a sequential autoanalyzer (SMA12/60; Technicon) by using Jaffe's method.

Statistical analysis

SAS version 8.02 for WINDOWS (SAS Institute Inc, Cary, NC) was used throughout the study. Women and men were analyzed separately. The chi-square test was used to compare dichotomous variables. To compare means between the 5 groups stratified by egg consumption, a one-way analysis of variance was used. To assess whether egg consumption affected total cholesterol concentrations, we performed an analysis of covariance by adjusting for age. Age-adjusted total cholesterol was determined.

The age-adjusted and multivariate-adjusted relative risks for all-cause or cause-specific mortality were calculated by using a Cox proportional hazard model. For multivariate analyses, age, serum creatinine, total cholesterol, blood glucose, body mass index, systolic and diastolic blood pressures, use of blood pressure–lowering drugs, cigarette smoking (never smoker; ex-smoker; current smoker, ≤20 cigarettes/d; and current smoker, >20 cigarettes/d), and alcohol intake (never drinker, ex-drinker, occasional drinker, and daily drinker) were entered as covariates. To rule out the possibility that subjects with a severe but subclinical disease might have affected the outcome, we performed the above Cox analyses after excluding subjects who died within the initial 5 y of follow-up. Tests of linear trends across groups with decreasing egg consumption were conducted by treating the median or representative values of egg consumption in the 5 categories (consumption per week: 21, 7, 3.5, 1.5, and 0.5 eggs, respectively) as continuous variables.

All P values were two-tailed, and $P < 0.05$ was considered significant. Data are presented as means ± SDs unless stated otherwise.

RESULTS

Baseline characteristics

Baseline characteristics in each egg consumption category for the women and the men are shown in **Table 1**. In both the women and the men, relatively few subjects (1.3–6.1%) were in the ≥2-eggs/d or seldom (ie, ≤1 egg/wk) group. Except for these 2 extreme categories, there were >1200 subjects in each category. In the women, those in the 2 extreme categories had significantly higher mean ages than did those in the other categories and were significantly more likely to have hypertension. Although similar tendencies were noted in the men, they were not as striking as those in the women.

Egg consumption and total cholesterol

Total cholesterol, age-adjusted total cholesterol, blood glucose, and serum creatinine concentrations and systolic and diastolic blood

TABLE 1
Baseline characteristics stratified by egg consumption among 5186 women and 4077 men with data in the NIPPON DATA80 database[1]

	Egg consumption					
Sex and characteristic	≥2/d	1/d	1/2 d	1–2/wk	Seldom	P^2
Women						
n	69	1393	1667	1742	315	
Age (y)	55.6 ± 12.9[3]	50.4 ± 12.7	49.3 ± 13.0	51.2 ± 13.3	55.4 ± 14.7	<0.0001
BMI (kg/m^2)	23.4 ± 3.8	22.7 ± 3.3	22.7 ± 3.2	23.0 ± 3.4	23.2 ± 3.7	0.0037
Hypertension (%)	49.3	38.3	37.0	43.7	51.8	<0.0001
Diabetes (%)	7.3	4.2	3.7	4.1	4.4	0.65
Daily drinker (%)	5.8	2.73	2.64	2.93	3.17	0.31
Current smoker (%)	11.6	7.5	7.8	10.6	10.5	0.0005
Men						
n	149	1364	1216	1204	144	
Age (y)	51.3 ± 12.6	51.0 ± 12.9	49.0 ± 12.7	50.6 ± 13.5	51.9 ± 13.9	0.001
BMI (kg/m^2)	22.2 ± 2.7	22.5 ± 2.9	22.5 ± 2.8	22.6 ± 2.9	22.4 ± 2.9	0.51
Hypertension (%)	49.3	38.3	37.0	43.7	51.8	<0.0001
Diabetes (%)	5.4	7.5	5.9	7.6	8.3	0.35
Daily drinker (%)	50.3	51.0	47.3	47.2	34.7	0.0001
Current smoker (%)	63.1	64.5	64.4	61.1	63.4	0.46

[1] NIPPON DATA80, National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980.
[2] Chi-square test for dichotomous variables and ANOVA for continuous variables.
[3] $\bar{x}$ ± SD (all such values).

pressures are shown in **Table 2**. In the women, a dose-response relation was noted between egg consumption and both total cholesterol and age-adjusted total cholesterol. No such relations were noted in the men, and total cholesterol and age-adjusted total cholesterol concentrations were almost the same in all categories.

Egg consumption and outcome: unadjusted outcome and multivariate Cox analyses

Unadjusted numbers of deaths due to all causes, stroke, IHD, and cancer for each category of egg consumption in the women and the men are shown in **Table 3**. Death rates are shown per 1000 person-years. In the women, all-cause, IHD, and cancer deaths differed significantly between the groups. In the men, no significant differences in outcome were noted. Because several baseline characteristics were different between the egg consumption categories, we performed multivariate analyses.

The results of age-adjusted and multivariate Cox analyses of associations between egg consumption and outcomes are shown in **Tables 4** and **5**. In the women, the multivariate-adjusted relative risk of all-cause death for those in the 1–2-eggs/wk category

TABLE 2
Baseline characteristics stratified by egg consumption among 5186 women and 4077 men with data in the NIPPON DATA80 database[1]

	Egg consumption					
Sex and characteristic	≥2/d	1/d	1/2 d	1–2/wk	Seldom	P^2
Women						
n	69	1393	1667	1742	315	
TCH (mmol/L)	5.23 ± 0.99[3]	4.97 ± 0.87	4.86 ± 0.84	4.85 ± 0.88	4.94 ± 1.01	<0.0001
aTCH (mmol/L)[4]	5.11 ± 0.10	4.98 ± 0.02	4.89 ± 0.02	4.83 ± 0.02	4.84 ± 0.05	<0.0001
Glucose (mmol/L)	7.39 ± 1.78	6.67 ± 1.83	7.11 ± 1.83	7.22 ± 1.94	7.28 ± 1.94	0.19
Creatinine (μmol/L)	78.7 ± 12.4	74.3 ± 11.5	74.3 ± 11.5	75.1 ± 21.2	76.9 ± 15.0	0.012
SBP (mm Hg)	139 ± 21	133 ± 21	132 ± 21	135 ± 22	140 ± 24	<0.0001
DBP (mm Hg)	83 ± 12	79 ± 12	79 ± 12	80 ± 12	81 ± 12	<0.0001
Men						
n	149	1364	1216	1204	144	
TCH (mmol/L)	4.73 ± 0.84	4.77 ± 0.83	4.78 ± 0.83	4.77 ± 0.86	4.77 ± 0.94	0.98
aTCH (mmol/L)[4]	4.76 ± 0.07	4.78 ± 0.02	4.78 ± 0.02	4.76 ± 0.02	4.79 ± 0.07	0.98
Glucose (mmol/L)	7.28 ± 1.89	7.28 ± 1.89	7.22 ± 2.11	7.33 ± 2.44	7.11 ± 1.72	0.0097
Creatinine (μmol/L)	90.2 ± 14.1	93.7 ± 26.5	93.7 ± 23.0	93.7 ± 15.0	95.5 ± 16.8	0.20
SBP (mm Hg)	139 ± 21	139 ± 20	137 ± 20	139 ± 22	142 ± 22	0.54
DBP (mm Hg)	84 ± 12	84 ± 12	83 ± 12	84 ± 12	84 ± 13	0.38

[1] NIPPON DATA80, National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980; TCH, total cholesterol; aTCH, age-adjusted total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[2] Chi-square test for dichotomous variables and ANOVA for continuous variables except aTCH, for which ANCOVA was used.
[3] $\bar{x}$ ± SD (all such values unless indicated otherwise).
[4] $\bar{x}$ ± SE.

TABLE 3
Unadjusted outcomes by egg consumption category during 14 y of follow-up of 5186 women and 4077 men with data in the NIPPON DATA80 database[1]

Sex and characteristic	Egg consumption					*P* (chi-square test)
	≥2/d	1/d	1/2 d	1–2/wk	Seldom	
Women						
n	69	1393	1667	1742	315	
Person-years	877	18 591	22 276	23 270	4058	
All-cause death [*n* (/TPY)]	13 (14.8)	149 (8.0)	166 (7.5)	175 (7.5)	59 (14.5)	<0.0001
Stroke death [*n* (/TPY)]	2 (2.3)	28 (1.5)	39 (1.8)	30 (1.3)	8 (2.0)	0.69
IHD death [*n* (/TPY)]	1 (1.1)	10 (0.5)	10 (0.4)	12 (0.5)	8 (2.0)	0.008
Cancer death [*n* (/TPY)]	5 (5.7)	40 (2.2)	43 (1.9)	45 (1.9)	15 (3.7)	0.043
Men						
n	149	1364	1216	1204	144	
Person-years	1934	17 652	16 008	15 610	1875	
All-cause death [*n* (/TPY)]	23 (11.9)	227 (12.9)	164 (10.2)	201 (12.9)	25 (13.3)	0.16
Stroke death [*n* (/TPY)]	1 (0.5)	37 (2.1)	32 (1.4)	37 (2.4)	5 (2.7)	0.52
IHD death [*n* (/TPY)]	0 (0)	9 (0.5)	11 (0.7)	17 (1.1)	2 (1.1)	0.23
Cancer death [*n* (/TPY)]	11 (5.7)	65 (3.7)	60 (3.7)	67 (4.3)	5 (2.7)	0.51

[1] NIPPON DATA80, National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980; TPY, 1000 person-years; IHD, ischemic heart disease.

was significantly lower than that for those in the 1-egg/d category. The relative risks of deaths from stroke, IHD, and cancer did not differ significantly between the egg consumption categories (Table 4). In the men, no significant differences in outcome between the egg consumption categories were noted (Table 5). The results of the multivariate Cox analyses after exclusion of the subjects who died within the initial 5 y of follow-up were not significantly different from those in Tables 4 and 5 (data not shown).

DISCUSSION

Egg yolk contains relatively high amounts of cholesterol, and this has led to the recommendation to limit egg intake to reduce serum cholesterol concentrations and hopefully prevent IHD. In fact, the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) recommended that at most 2 egg yolks should be consumed per week (1). Although several metabolic ward studies showed that dietary cholesterol is a major determinant of serum cholesterol concentrations (2, 3), other studies failed to detect changes in serum total cholesterol concentration when egg was added to diets that already contained moderate amounts of cholesterol (4–7). Furthermore, epidemiologic studies in the United States did not detect any associations between egg consumption and serum cholesterol concentrations or cardiovascular events (11, 12). However, in geographic areas where egg consumption makes a greater contribution to total dietary cholesterol intake than in the United States, the results may be different (13–15). The studies by Dawber et al (11) and Hu et al (12) reported that egg consumption in the United States accounts for 26–32% of total dietary cholesterol intake. In contrast, a study in

TABLE 4
Relative risks and 95% CIs of outcomes by egg consumption category in Cox analyses of women with data in the NIPPON DATA80 database[1]

	Egg consumption					*P* for trend
	≥2/d (*n* = 69)	1/d (*n* = 1393)	1/2 d (*n* = 1667)	1–2/wk (*n* = 1742)	Seldom (*n* = 315)	
Age adjusted						
All-cause death	1.57 (0.89, 2.76)	1	1.0 (0.81, 1.23)	0.82 (0.66, 1.01)	0.99 (0.74, 1.33)	0.02
P	0.12		0.97	0.06	0.93	
Stroke death	1.51 (0.36, 6.33)	1	1.42 (0.88, 2.30)	0.85 (0.51, 1.41)	0.76 (0.35, 1.66)	0.18
IHD death	1.68 (0.22, 12.9)	1	0.82 (0.36, 1.88)	0.76 (0.34, 1.66)	1.70 (0.70, 4.14)	0.90
Cancer death	2.19 (0.87, 5.53)	1	0.97 (0.64, 1.48)	0.84 (0.55, 1.27)	1.18 (0.65, 2.12)	0.10
Multivariate adjusted[2]						
All-cause death	1.48 (0.84, 2.61)	1	1.0 (0.81, 1.24)	0.78 (0.63, 0.96)	0.97 (0.72, 1.32)	0.02
P	0.17		0.98	0.02	0.86	
Stroke death	1.22 (0.29, 5.17)	1	1.46 (0.89, 2.4)	0.79 (0.47, 1.33)	0.78 (0.35, 1.73)	0.23
IHD death	1.27 (0.16, 9.80)	1	0.78 (0.35, 1.82)	0.64 (0.28, 1.44)	1.42 (0.56, 3.62)	0.71
Cancer death	2.36 (0.93, 5.98)	1	0.93 (0.61, 1.41)	0.76 (0.52, 1.20)	1.18 (0.65, 2.12)	0.06

[1] NIPPON DATA80, National Integrated Project for Prospective Observation of Non-communicable Disease And its Trends in the Aged, 1980; IHD, ischemic heart disease.

[2] Age, serum creatinine, total cholesterol, blood glucose, BMI, systolic and diastolic blood pressures, use of blood pressure–lowering drugs, cigarette smoking, and alcohol intake were entered as covariates for multivariate analyses.