Table 5. Results of Multivariate Analysis for Predictors of N1 Disease

Variable	Risk Ratio	95% Confidence Interval	*p* Value
CEA (continuous variable; ng/mL)	1.092	1.029–1.158	0.0037
Radiologic classification (type 1–4 vs 5–6)	0.074	0.010–0.554	0.0112

CEA = carcinoembryonic antigen.

carcinoma of the lung. It is probably safe to say that segmental resection or wide wedge resection is sufficient for such tumors because of their minimally invasive nature. Type 1 tumor is also known as pure GGO or simple GGO [18]. Among 22 type 1 tumors, there was no lymph node metastasis, and pathologic findings showed minimal invasion. There were 15 (68%) tumors that were equivalent to the type A or B tumors of Noguchi and colleagues [6], ie, roughly bronchioloalveolar carcinoma. Type 2 tumor is denser than type 1 tumor on thin-section CT scan. This tumor is not a solid tumor because we can see the underlying bronchovascular structure. No lymph node metastasis was noted, and 11 tumors were similar to the type A or B tumors of Noguchi and colleagues [6]. The difference in their density is probably related to the difference in the amount of air contained in the tumor, ie, differences in alveolar space histologically. Type 3 tumor is also known as GGO halo. One tumor had metastasized to the intrapulmonary lymph node, ie, N1 node, but 15 tumors were still diagnosed as being equivalent to the type A or B tumor of Noguchi and colleagues [6]. Type 4 tumor is actually defined by our original definition. This tumor consists of a mixture of GGO and a solid part containing air, roughly air-bronchogram. There was no lymph node metastasis and no lymphatic invasion. Basically, lung adenocarcinoma in the above four types is thought to be "minimally invasive" adenocarcinoma. A limited anatomic resection of the lung could be the standard surgical procedure for such tumors in the near future.

Type 5 and 6 tumors are considered to exhibit a "solid" course. Lymph node metastasis was found in roughly 5% of type 5 tumors, and 27% of type 6 tumors. Traditionally, lymph node metastasis is found in approximately 15% of small adenocarcinoma 2.0 cm or less in size. According to our results, however, lymph node metastasis was found mostly in type 6, which meant that if peripheral lung adenocarcinoma showed GGO on thin-section CT, the probability of lymph node metastasis was less than 5%. These "solid" tumors could be divided into several subgroups by means of positron emission tomography. If the solid tumors show positive results by positron emission tomography, they may be associated with a high frequency of lymph node metastasis and a poor prognosis.

One of the important objectives of this study is to determine the indication for limited surgical resection for lung adenocarcinomas. From this concept, the classification became simpler if the classification was composed with groups, ie, types 1 through 4 and types 5 and 6. If a tumor belongs to types 1 through 4, the patient would be a candidate for limited surgical resection, whereas a tumor belonging to group 5 or 6 warrants major lung resection with systematic lymph node dissection necessary. However, we believe the six classifications proposed in this study remain important for the surgeon to plan for the management of peripheral lung cancer. For instance, most of the type 1 tumors are bronchioloalveolar carcinoma, and some of them might be indolent tumors. On the contrary, type 2 tumors tend to be adenocarcinoma with invasive foci pathologically and grow in size. Actually we made a plan for a prospective follow-up study for type 1 tumors, not for type 2 tumors. Thus, clinical strategy depends on the six classifications, and we hope to leave the classification intact.

As to the surgical indications for pure GGO tumors, we resected the tumor if it is stable or increased in size. However, from our data, tumors belonging to type 1 could be bronchioloalveolar carcinoma, and are sometimes indolent. Thus, recently we just monitor such type 1 tumors without surgical interventions if the radiologic maximal

Table 6. Review of Literature Regarding Proportion of Ground-Glass Opacity as Radiologic Prognostic Factors in Adenocarcinoma of the Lung

Authors	Year	No.	Cases	Methods	Good Prognosis	Analysis
Jang et al. [9]	1996	14	...	...	Focal area of GGO	Univariate
Aoki et al. [4]	2001	127	Ad, cT1	Dimension	GGO > 0.5	Univariate
Kodama et al. [10]	2001	104	Ad, 2 cm or less	Visual	GGO > 0.5	Multivariate
Takamochi et al. [19]	2001	269	Ad, peripheral	TDR	TDR & CEA	Multivariate
Kim et al. [11]	2001	224	Ad, cT1	Visual	GGO extent	Univariate
Matsuguma et al. [12]	2002	111	Ad, cIA	Visual	GGO > 0.5	Univariate
Takashima et al. [15]	2002	64	Ad, 2 cm or less	CT	GGO > 0.57	Multivariate
Suzuki et al. [14]	2002	69	Ad, cIA	Dimension	GGO > 50%	Univariate
Okada et al. [17]	2003	167	Ad, cT1	TDR	TDR > 0.5	Multivariate
Ohde et al. [13]	2003	98	Ad, cT1	Dimension	GGO > 50%	Univariate

Ad = adenocarcinoma; CEA = carcinoembryonic antigen; cT1 = clinical T1; GGO = ground-glass opacities; TDR = tumor disappearance ratio.

tumor dimension is less than 15 mm. If radiologic findings suggest the tumor as lung cancer, preoperative CT-guided fine-needle biopsies are not always performed because of the high rate of a false-negative result for GGO tumors.

In conclusion, a new radiologic classification of small-sized adenocarcinoma of the lung has been proposed. Because this is the retrospective study, there may be numerous levels of bias. Therefore, we are planning to perform a prospective study of the management of peripheral small adenocarcinoma of the lung. Using the classification, we can easily classify peripheral adenocarcinoma of the lung into six categories, and the classification is significantly associated with pathologic prognostic factors. Future treatment strategies for small-sized adenocarcinoma of the lung may be based on this new radiologic classification.

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INVITED COMMENTARY

Anatomical resection is the standard treatment for early stage nonsmall cell lung cancer. As radiographic scanning methods are improving and we are identifying cancers at smaller sizes than previously recognized, the issue of limited resection for small peripheral cancers is being reinvestigated. Should small size on computed tomography (CT) be the only criteria with which to determine the type of resection (ie, anatomical versus limited [wedge]) to be performed? The answer would be no, according to the article by Suzuki and colleagues [1]. This study is a retrospective review of a single institutional experience in 349 chemotherapy–radiotherapy naive patients with small, single peripheral lung primary adenocarcinomas during a 4-year period of time from 1999 to 2003 to evaluate a new radiographic classification that may assist in the future management of patients. From their classification, in essence a radiologic Noguchi classification [2], they were able to identify a group of patients who might be best treated with limited resection. In their series, the 42 patients with either N1 or N2 disease seemed to have a greater solid component and less ground glass opacification (GGO) features than those who did not have those features. The authors concluded that their classification may be a useful evaluation system for future trials.

Some articles raise more questions than answers, as does this article. Clinicians should not be tempted to follow the authors' implications (given the retrospective design of this study) that thin-section CT peripheral lung

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Genetic Classification of Lung Adenocarcinoma Based on Array-Based Comparative Genomic Hybridization Analysis: Its Association with Clinicopathologic Features

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Abstract The array-based comparative genomic hybridization using microarrayed bacterial artificial chromosome clones allows high-resolution analysis of genome-wide copy number changes in tumors. To analyze the genetic alterations of primary lung adenocarcinoma in a high-throughput way, we used laser-capture microdissection of cancer cells and array comparative genomic hybridization focusing on 800 chromosomal loci containing cancer-related genes. We identified a large number of chromosomal numerical alterations, including frequent amplifications on *7p12*, *11q13*, *12q14-15*, and *17q21*, and two homozygous deletions on *9p21* and one on *8p23*. Unsupervised hierarchical clustering analysis of multiple alterations revealed three subgroups of lung adenocarcinoma that were characterized by the accumulation of distinct genetic alterations and associated with smoking history and gender. The mutation status of the *epidermal growth factor receptor* (*EGFR*) gene was significantly associated with specific genetic alterations and supervised clustering analysis based on *EGFR* gene mutations elucidated a subgroup including all *EGFR* gene mutated tumors, which showed significantly shorter disease-free survival. Our results suggest that there exist multiple molecular carcinogenesis pathways in lung adenocarcinoma that may associate with smoking habits and gender, and that genetic cancer profiling will reveal previously uncharacterized genetic heterogeneity of cancer and be beneficial in estimating patient prognosis and discovering novel cancer-related genes including therapeutic targets.

Lung cancer is one of the most lethal and increasing cancers in Western countries as well as in Japan (1). Lung cancer is histopathologically divided in two subgroups, small cell lung carcinoma and non-small cell lung carcinoma, and lung adenocarcinoma comprises >40% of the latter (1).

Previous genetic analyses using allelotyping, comparative genomic hybridization (CGH), or the candidate gene approach revealed many genomic (genetic and epigenetic) alterations of tumor suppressor genes (such as *p53*, *p16^{INK4a}*, *FHIT*, *LKB1*, and *PTEN*) and oncogenes [such as *K-ras*, *B-RAF*, *MYC*, *epidermal growth factor receptor* (*EGFR*), and *ERBB2*] as well as many chromosomal imbalances (such as on 3p, 8p, 9p, 17p, 18q, and 19p) in lung adenocarcinomas (2–10). However, overall understanding of genomic alterations in lung adenocarcinomas is far from complete and analysis of the relationship between the overall profile and combinations of genetic alterations with clinicopathologic parameters is still lacking. Recently, genome-wide gene expression analyses have uncovered a novel dimension of cancer profiling and helped define the nature of the heterogeneous subgroups of lung adenocarcinoma, each of which shows distinct tumor histology and patient prognosis (11–14). However, it is unclear whether there exist multiple genomic pathways in lung adenocarcinoma because of the lack of a genome-wide view of genetic alterations. It is clinically important to examine the correlations of certain molecular-genetic pathways with cancer cell traits relating to patient prognosis or chemotherapy sensitivity because it is possible that genetic alteration profiling may predict tumor recurrence/metastasis or sensitivity to molecular-target therapies as well as mRNA or protein expression profiles do (15–17).

The recently developed array-based CGH method using microarrayed bacterial artificial chromosome clones allows high-resolution analysis of genome-wide copy number changes in various tumors (18, 19). To define and analyze the genetic alterations of lung adenocarcinoma in a more detailed way,

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we used the array CGH technique and laser-capture microdissection of cancer cells, a combination that we have successfully used in other tumor types (20).[9] Using these two powerful methodologies enabled us to detect a considerable number of novel chromosomal numerical alterations in primary lung adenocarcinoma. Unsupervised and supervised hierarchical clustering analyses of genome-wide genetic alterations revealed the presence of heterogeneous groups of lung adenocarcinoma, which are characterized by specific combinations of genetic alterations, varying *EGFR* gene mutation status, and tumor recurrence rates.

Materials and Methods

Patient materials. Surgical specimens of 55 lung adenocarcinoma patients who had been diagnosed and undergone operation between June 2001 and May 2002 at the National Cancer Center Hospital were examined. Fragments of tumor and corresponding normal lung tissue were taken immediately after surgery, fixed with 100% methanol, and embedded in paraffin. This study was approved by the institutional review boards of the National Cancer Center. The clinicopathologic data of the patients are shown in Table 1.

Laser-capture microdissection and whole-genome amplification. Laser-capture microdissection was done using LM200 (Arcuturus, Mount View, CA) as described (21). Only cancer cells were microdissected and lymphocytes, fibroblasts, and endothelial cells were carefully excluded. Corresponding normal lung epithelial cells were similarly microdissected and used as reference. To amplify the genomic DNA fragments, we used an adaptor-ligated whole-genome PCR as previously reported (22).

Array-based comparative genomic hybridization. This study used a custom-made CGH array called "MCG CancerArray-800 ver.2," which consists of 800 duplicated bacterial artificial chromosome clones corresponding to various chromosomal loci that have been reported or considered to be altered in various human cancers (20, 23). Details of hybridization procedures have been previously reported (20). Sixteen-bit fluorescence intensity TIF images were obtained using a scanner (FLA8000, Fuji Film, Tokyo, Japan) and analyzed using GenePix Pro 5.0 (Axon Instruments, Inc., Foster City, CA). Thresholds for chromosomal gain (ratio >1.25) and loss (ratio <0.75) were determined by "normal versus normal experiments" (23, 24). We also validated our array CGH data by other methods. Loss of the *17p13* locus was confirmed by loss of heterozygosity of the *p53* gene, which is located within that bacterial artificial chromosome using a microsatellite marker (TP53CA). Gene amplification of a representative gene, *cyclin D1*, was validated by fluorescence *in situ* hybridization analysis (24). We applied multiplex ligation-dependent probe amplification (MLPA) to validate ourarray data. Copy number alterations of multiple loci were analyzed using MLPA-SALSA kit (MRC-Holland, Amsterdam, the Netherlands) as per the recommendation of the manufacturer (25). Size and quantity of PCR products were calculated by Gene Mapper software (Version 3.5, Applied Biosystems, Tokyo, Japan) and copy number was determined by the ratio to the average of five normal control experiments.

Mutational analysis. We amplified exons 18, 19, 21, and 23 of the *EGFR* gene; exons 2 and 3 (covering codons 12, 13, and 61) of the K-*ras* gene; exons 20 and 21 of the *ERBB2* gene; and exons 10, 14, 16, 17, 18, 19, and 20 of the *MET* gene from microdissected tumor and corresponding normal DNA samples with PCR using High Fidelity Taq polymerase (Roche, Mannheim, Germany) and appropriate primers (primer sequences are available on request). All PCR products were purified and analyzed by sequencing. PCR products showing deletions were subcloned in TA-vector (Invitrogen, Carlsbad, CA) and sequenced.

[9] Unpublished data. Shibata T, Hosoda F, Ohki M, Hirohashi S.

Table 1. The clinicopathologic characteristics and oncogenic mutation profiles of patients and tumors

	No. cases	Frequency (%)
Total no. patient	55	
Mean age (range)	62.3 (35-79)	
Gender		
Male	28	50.9
Female	27	49.1
Smoking history		
Never	27	49.1
Former	10	18.2
Current	18	32.7
Tumor differentiation		
Well	20	36.4
Moderate	25	45.5
Poor	10	18.1
Stage*		
I (IA and IB)	24	43.6
II (IIA and IIB)	6	10.9
III (IIIA and IIIB)	21	38.2
EGFR mutation	26	47
Exon 18 G719S	1	1.8
Exon 19 Del746-750	8	14.5
Exon 19 Del747-752	2	3.6
Exon 19 Del747-752insS	1	1.8
Exon 21 L858R	14	25.5
K-*ras* mutation	6	11
Codon 12	4	7.3
Codon 13	1	1.8
Codon 61	1	1.8

*Clinical stage of four cases was not evaluated.

Immunohistochemical analysis. Four-micrometer sections of formalin-fixed, paraffin-embedded specimens of lung adenocarcinoma were stained with an anti-MET mouse monoclonal antibody (×100 dilution, Zymed, San Francicso, CA) as the suppliers recommended.

Statistical analysis. Two-dimensional hierarchical clustering analysis of the samples and signal ratios was done using the Impressionist (Gene Data, Basel, Switzerland) and GeneMaths (Applied Maths, Sint-Martens-Latem, Belgium) software programs as described (26, 27). Data were standardized by dividing by the root means and dendrograms were produced using the Pearson Correlation algorithm. For supervised clustering, we first selected loci that were significantly different between *EGFR* wild-type and mutated tumors based on the average ratio by Student's *t* test. We then used a machine-learning method, in which the leave-one-out cross-validation was done with all combinations of loci and multiple independent classifier algorithms, and selected 46 loci that could discriminate *EGFR* mutation status most accurately to classify the tumors. The Kaplan-Meier method was used to estimate the probability of disease-free survival. Cox proportional hazards regression model and multivariate analysis were done to detect the association between the presence of chromosomal alterations and disease-free survival. Log-rank analysis was used to assess the significance of the difference between subgroups.

Results

Array-based comparative genomic hybridization analysis of primary lung adenocarcinoma. We analyzed 55 cases of lung

adenocarcinoma by array-based CGH and the chromosomal alteration profiles of 800 loci are shown in Fig. 1. We identified 32 loci that were lost in >40% of cases (Table 2). Among them, the *9p21* locus containing the $p16^{INK4a}$ gene and the *17p13.1* locus containing the *p53* gene were lost in 54% and 40% of analyzed cases, respectively. We found homozygous deletions of three loci, including two on *9p21* and one on *8p23.3*. We also identified 19 loci that were gained in >50% of cases (Table 3) and recurrent (>4 cases) amplifications (>4 copies) on *12q14-15* (9 of 55, 16.3%) followed by *7p12.3* (5 of 55), *11q13* (5 of 55), *17q12* (5 of 55), *1p36.1* (4 of 55), *1q21* (4 of 55), *5p15* (4 of 55), *7q31* (4 of 55), *8q24* (4 of 55), *14q12* (4 of 55), and *17q21.2* (4 of 55). These included genes previously reported to be amplified in lung cancer, such as the *cyclin D1* (*11q13*), *EGFR* (*7p12.3*), and *ERBB2* (*17q21.2*) genes (28, 29). We further validated copy number alterations on *8q24.3*, *17q21.2*, *3p21*, and *17p13.1* by MLPA method. Chromosomal copy number changes (both gains and losses) detected by array CGH corresponded to those by MLPA (Fig. 1C).

Unsupervised hierarchical clustering of array comparative genomic hybridization data. To examine whether there exist multiple carcinogenesis pathways in lung adenocarcinoma, we attempted two-dimensional hierarchical profiling of the chromosomal alterations detected. We first plotted the number of loci showing various incidences of alterations and found that there exist two peaks (loci altered in 10-15% and 20-25% of cases; Fig. 1D). We assumed that alterations appearing in <20% of cases reflect mostly random alterations as observed in genome-wide allelotyping analyses (30), whereas alterations affecting >20% of cases probably represent nonrandom (cancer-specific) alterations. Therefore, to exclude random changes that may be caused by the intrinsic genetic instability of cancer, we selected the loci that were affected in >25% of analyzed cases (397 loci in total) and did the unsupervised hierarchical clustering analysis. When analyzed by using loci affected in >5% and 15% of cases, we obtained almost the same classification as described below (data not shown).

Our hierarchical clustering yielded three distinct subclasses of primary lung adenocarcinoma (clusters A, B, and C shown in Fig. 1E). Cluster B exhibited significantly fewer genetic alterations (losses and gains) in all examined clones than the other two clusters; the average number of alterations (losses, gains, total) in cluster A was 102, 141, and 244, respectively; in B 43, 81, and 125; and in C 85, 131, and 216 (A versus B: $P < 0.0001$; B versus C: $P < 0.0001$). Frequencies of the various lost or gained loci were significantly different among these three cluster groups ($P < 0.01$). Cluster A was characterized by gains on *1p32-26*, *4p16.3*, *11p15*, *12q13-14*, *16p11.2-13.3*, *17q11.1-25*, *19q13.2*, *20p11*, *20q11.2*, and *22q12.2* and losses on *1p22*, *6q26*, *10q24-26*, *13q22.1-34*, *15q21-25*, and *18p11.2*. Cluster C significantly showed gains on *5p12-14.3*, *7p12.3-21.1*, *7q22*, *7q31*, *8q12-21*, and *14q11-24*, and losses on *1q23.3-41*, *10q22.1*, and *Xq*. Some loci were similarly altered in both clusters A and C, including losses on *3p21-24*, *6q26*, *8q24.3*, *9q21*, *10p15*, *10q11*, *10q26*, *15q21.1*, *15q26.1*, and *19p13.3* (containing *LKB1*), and gains on *5p15*, *6p21*, *7p21-22*, *7q21*, *8q21*, *8q22-24* (containing *MYC*), *9q21-22*, *11q13* (containing *cyclin D1*), and *20q13.1*. Losses on *3p14* (containing *FHIT*), *8p22-23.3*, *9p21* (containing $p16^{INK4a}$), *13q11-34*, *17p13.1* (containing *p53*), *18q21*, and gains on *1q21-23*, *1q42*, *7p15*, *17q12*, *17q21.2*, and *17q25* were observed in all subgroups with similar frequency. Two alterations (a gain on *19q13.1* and a loss on *22q12.2*) were more frequently observed in cluster B than in clusters A and C. The above classification into cluster groups showed significant correlation with the patients' smoking history ($P < 0.01$) and gender ($P < 0.001$); cluster A frequently contained female patients without any smoking history (female: 17 of 20 cases and never smoker: 14 of 20 cases), whereas cluster B included male patients with current or former smoking history (male: 11 of 15 cases and smoker: 11 of 15 cases). Cluster C included more male patients (male: 14 of 20 cases), but showed no significant association with smoking history (smoker: 11 of 20 cases). No significant differences were observed between the groups with regard to other clinical features (histologic differentiation, clinical stage, and disease-free survival). Multivariate analysis revealed that two chromosomal alterations showed significant association with disease-free survival: a loss on 13q14.1 ($P = 0.01$, hazard ratio, 3.21; 95% confidence interval, 1.30-7.91) and a gain on 8q24.2 ($P = 0.02$, hazard ratio, 2.92; 95% confidence interval, 1.16-7.37).

It has been reported that somatic mutations of the K-*ras*, *EGFR*, and *ERBB2* genes are frequent in lung adenocarcinoma (2, 8-10, 31-33). We attempted to determine the correlation of these oncogenic mutations with the above classification. We sequenced exons covering the kinase domain of the *EGFR* gene and found somatic mutations in 26 cases (47%; Table 1). *EGFR* mutations were more frequently observed in never-smoker patients ($P < 0.001$) and in the A and C cluster groups ($P = 0.01$ and 0.02). We detected K-*ras* activating mutations in six cases (11%; Table 1) and *EGFR* and K-*ras* mutations were mutually exclusive in our cases as reported by others (31, 32). No mutation in the reported exons of the *ERBB2* gene was detected.

Supervised clustering analysis revealed correlation of EGFR gene mutation with specific genetic alterations. Tumors with *EGFR* mutations showed significantly more genetic alterations (losses and gains) than those without *EGFR* mutations; the average numbers of alterations (losses, gains, total) were 68, 110, and 178 in *EGFR* wild-type tumors and 93, 134, and 227 in *EGFR* mutated tumors, respectively (wild-type versus mutated $P = 0.01$, 0.03, and 0.01). We found 58 loci that showed significant differences in the frequency of copy number alterations between *EGFR* wild-type and mutated tumors. To further examine the genetic profile of *EGFR* mutated tumors, we classified lung adenocarcinomas based on their *EGFR* mutation status with the use of supervised hierarchical clustering. We preformed a machine-learning method with leave-one-out cross-validation and selected 46 loci that could discriminate *EGFR* mutation status. *EGFR* wild-type and mutated tumors were clustered in distinct groups using the ratios of 46 selected loci (Fig. 2A). Tumors carrying K-*ras* mutations were segregated from the *EGFR* mutant branch (Fig. 2A). Interestingly, some cases without *EGFR* mutation were clustered with the mutant branch, and tumors carrying *EGFR* mutations were separated in two subbranches (EGFR-MUT-A and EGFR-MUT-B; Fig. 2A). One branch (EGFR-MUT-A) was characterized by amplification of *12q14* or *1p36.1*, whereas the other (EGFR-MUT-B) contained frequent amplification of *7p12.3* (containing the *EGFR* gene), *1q44-23*, *5p12*, *14q31*, and *16p13.3*. Poorly differentiated tumors were significantly ($P = 0.01$) segregated in the EGFR-MUT-B subgroup (EGFR-MUT-A: 1 of 15 cases and EGFR-MUT-B: 5 of 21 cases).

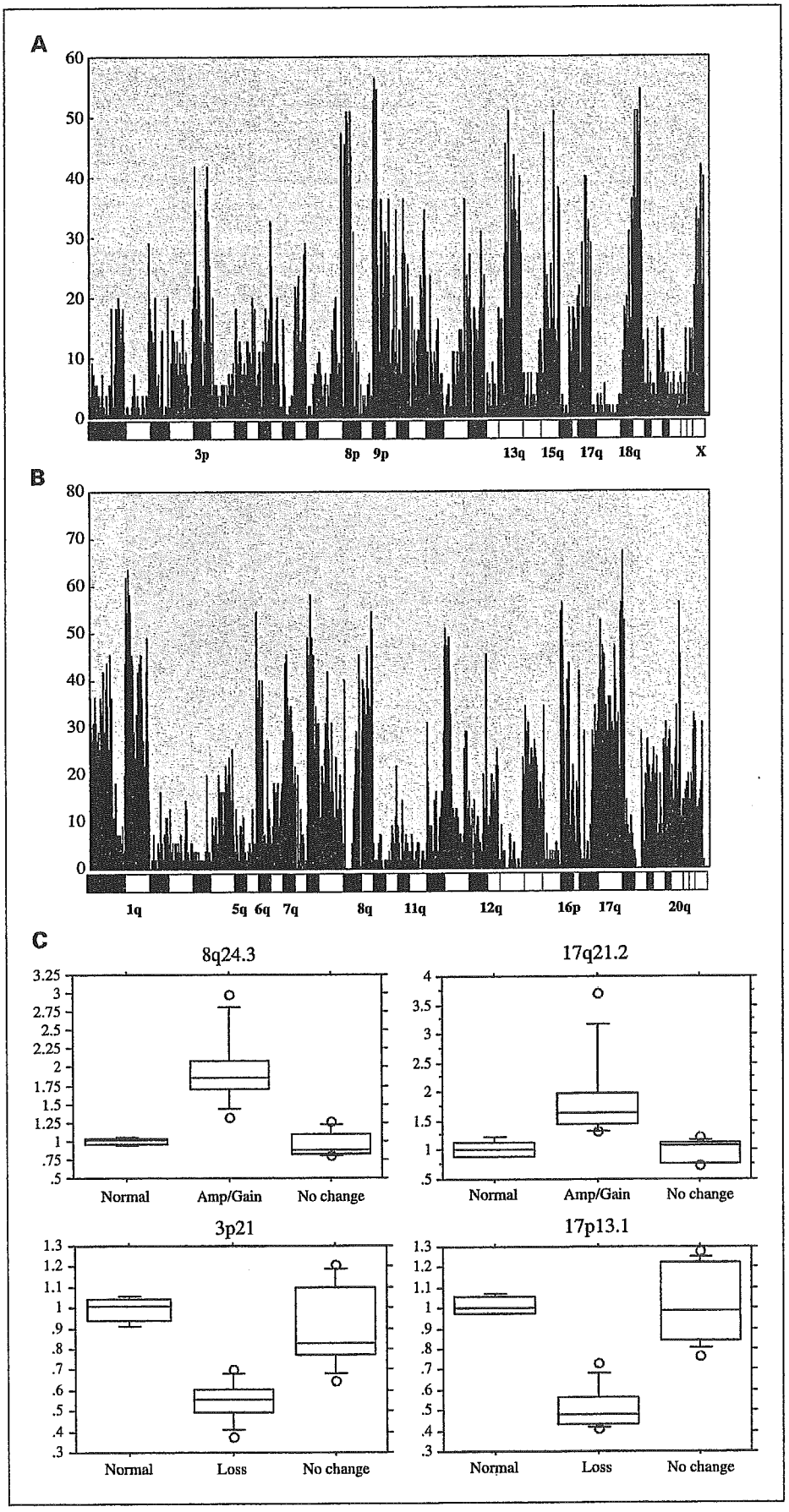

Fig. 1. Copy number alteration profiles of primary lung adenocarcinoma. Frequencies (%) of chromosomal losses (*A*) and gains (*B*) detected by array CGH analyses are plotted from chromosome 1p (*left*) to Y (*right*). Each chromosome is represented by underlining boxes (short and long arms are indicated by closed and open boxes, respectively). Chromosomal arms that contain frequent losses or gains are also indicated. *C*, validation of array CGH analysis. Distributions of copy number on *8q24.3*, *17q21.2*, *3p21*, and *17p13.1* loci detected by MLPA method in five normal samples (*Normal*), tumors with alterations detected by array CGH (*Amp/Gain* or *Loss*), and tumors without alteration by array CGH method (*No change*) were shown.

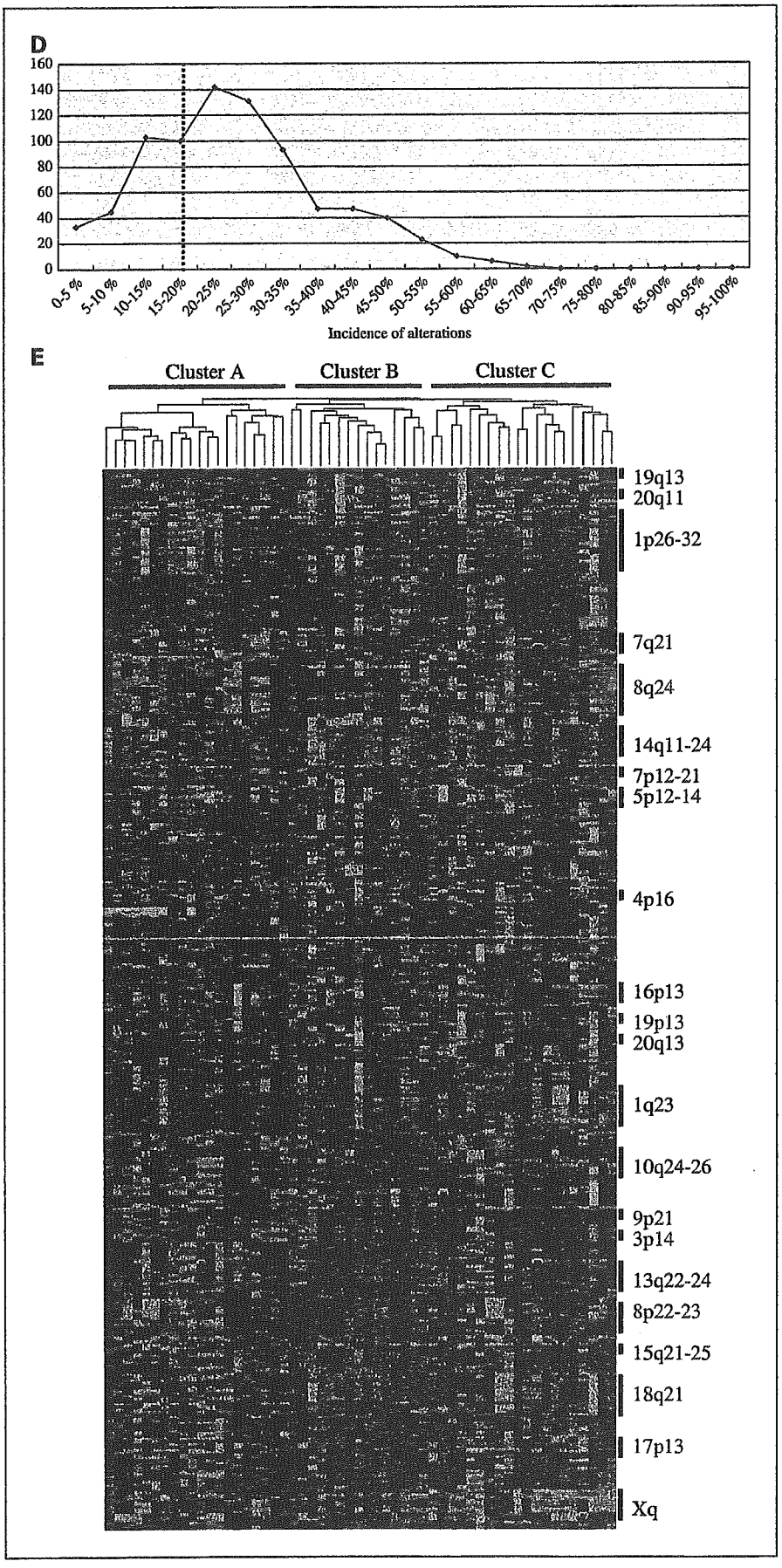

Fig. 1. *Continued*. *D* and *E*, genetic classification of lung adenocarcinoma. *D*, number of loci as a function of the percentage of altered loci in lung adenocarcinoma. Two modes, which can be separated by the vertical dotted line, can be observed. Loci within the first mode (altered in <20% of cases) were considered random, whereas those in the second mode (altered in >20% of cases) were considered pathogenic. *E*, unsupervised genetic profiling of lung adenocarcinoma. Fifty-five lung adenocarcinomas were clustered hierarchically on the basis of copy number changes in 397 loci. Multiple lost (*green*) or gained (*red*) loci are observed in individual tumors. Overall pattern of standardized gene copy numbers and the cluster tree of individual tumors are shown. Tumors were clustered in three subgroups (clusters A, B, and C). Loci that are specific for (*black letters*) or shared with (*blue letters*) each subgroup are indicated on the right side.

The *EGFR* wild-type tumors that were clustered with the EGFR-MUT branch led us to hypothesize that aberrant activation of tyrosine kinases other than *EGFR* have an effect equivalent to that of *EGFR* mutations in these tumors. We examined the copy number changes of loci containing oncogenic receptor-type tyrosine kinases (FGFR1, FGFR2, FGFR3, PDGFR, KIT, MET, ERBB2, FLT3, NTRK1, and NTRK3) detected by our arrays. We found that amplification of a locus containing the *MET* gene (*7q31*) was observed in *EGFR* wild-type tumors (three of four amplified cases; a representative case was shown in Fig. 3A) and that these tumors were clustered with the EGFR-MUT branch (Fig. 2A). Overexpression of MET protein was immunohistochemically detected in 24% (13 of 55) of cases including all cases with amplification of the *MET* gene (Fig. 3B), although there was no significant association between MET overexpression and the clustering. To determine whether somatic mutations of this kinase also occur in lung adenocarcinoma, we sequenced the exons of the *MET* gene, which have been reported to exhibit activating mutations in various tumors (34–36); however, no mutation was found in any of examined 55 cases.

We further examined whether this classification is of clinical significance. Kaplan-Meier plots showed a statistically significant difference in disease-free survival between the two groups (EGFR-WT and EGFR-MUT; log-rank analysis, $P = 0.01$; Fig. 2B) although *EGFR* mutation status alone did not (log-rank analysis, $P = 0.06$; data not shown).

Table 2. Loci frequently lost in primary lung adenocarcinoma

Chromosomal location	Covered candidate gene	Chromosomal loss in lung adenocarcinoma (%)
9p22	*MLLT3*	56.4
9p21	*p16^{INK4a}* *	54.5
9p21	*TEK*	54.5
18q23d	*CTDP1*	54.5
9p23	*GASC1*	52.7
9p21.3	*MTAP*	52.7
15q25	*NTRK3*	50.9
13q14.1	*FKHR*	50.9
18q21	*SMAD4**	50.9
8p22	*NAT2*	50.9
18q21.3	*PI5*	50.9
18q21	*GRP*	50.9
18q22	*BCL2*	50.9
8p22	*LZTS1**	49.1
15q12	*SNRPN*	47.3
8p23.3	*D8S504*	47.3
18q21.3	*SCCA1*	47.3
8p22	*N33*†	45.5
13q11-12	*FGF9*	45.5
8p22-11	*NRG1*	43.6
13q22.1	*KLF12*	43.6
Xq28	*MAGEA2*	41.8
3p24.3	*THRB**	41.8
3p14.2	*FHIT**	41.8
8p22-21.3	*DLC1*†	41.8
8p23.1	*AAC1*	41.8
17p11.2	*RH68621*	40
13q14.1	*LCP1*	40
8p22-8p21	*TNFRSF10B*	40
13q33	*EFNB2*	40
17p13.1	*RCV1*	40
18q21.3	*FVT1*	40

*Loss of heterozygosity or mutations, and †aberrant expression was previously reported in lung cancer (3–5).

Table 3. Loci frequently gained in primary lung adenocarcinoma

Chromosomal location	Covered candidate gene	Chromosomal gain in lung adenocarcinoma (%)
17q25	*MAFG*	67.3
1q21	*MUC1**	63.6
1q21	*MCL1**	61.8
7p21	*IL6*	58.2
1q21	*ARHGEF2*	58.2
16p13.3	*ABCA3*	56.4
17q11	*ITGB4*	56.4
20q13	*Livin-2*	56.4
5p15	*TERT*	54.5
8q24	*GLI4*	54.5
16p13.3	IGFALS	54.5
17q24-25	*GRB2*	54.5
1q21	*AF1Q*	54.5
1q23.1	*PMF1*	54.5
12q24	*stSG8935*	52.7
17q12	*PPARBP*	52.7
17q25	*Survivin**	50.9
8q24	RECQL4	50.9
11q12-13	RELA	50.9

*Aberrant expression was previously reported in lung cancer (3–5).

Discussion

This study is the first high-resolution copy number analyses of primary lung adenocarcinoma by array CGH method. To extract common and specific genetic alterations, we collected and analyzed 55 cases of primary lung adenocarcinoma by combining the array-based CGH analysis with laser-capture microdissection of tumor cells. Importantly, our results were validated by the elucidation of frequent alterations of previously reported cancer-related genes in lung adenocarcinoma, including losses on the *p16^{INK4a}*, *p53*, and *FHIT* loci, and amplifications on the *cyclin D1* and *EGFR* loci. Moreover, we elucidated novel frequent alterations in small chromosomal regions such as losses on *13q11-14* and *15q12-25* and gains on *17q25*, *1q21*, and *16p13.3*, which have not been detected by previous studies. Novel recurrent amplification, which may be a landmark for the existence of oncogenes, was also detected on loci, including *1p36.1*, *1q21*, *5p15*, *12q14-15*, and *14q12*. We also found a homozygous deletion on *8p23.3* accompanied with frequent chromosomal loss and identification of candidate tumor suppressor genes in this locus is in progress.

It has been argued that there are distinct subclasses of lung adenocarcinoma by histopathologic observations and recent gene expression profilings (2, 13). Girard et al. (30) reported the possibility of classification of lung cancer by genome-wide allelotyping although their study only examined lung cancer cell lines and could not discriminate between copy number gain and loss. In our study, we analyzed primary lung adenocarcinoma and used unsupervised hierarchical cluster analysis to identify three groups of lung adenocarcinoma based on their distinct genetic changes. Among them, two subclasses (clusters A and C, 20 of

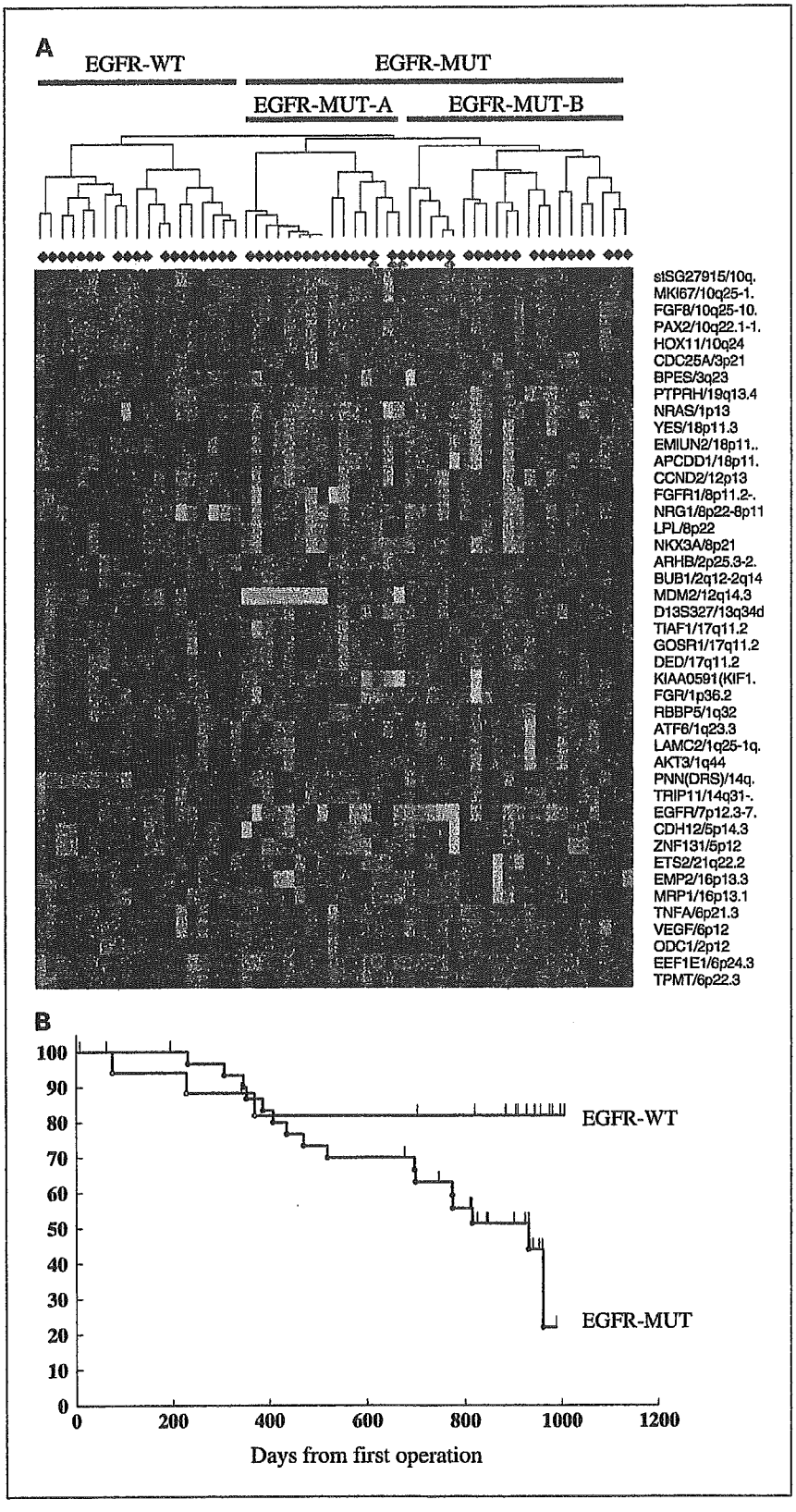

Fig. 2. Supervised genetic profiling of *EGFR* mutation – related loci. *A*, Hierarchical clustering determined copy number change patterns against 46 loci that were identified using the training testing, cross-validation analysis. Lost (*green*) or gained (*red*) loci were indicated in individual tumors. Tumors are classified into two branches (EGFR-WT and EGFR-MUT). All *EGFR* mutated tumors (*red spot*) are clustered in the EGFR-MUT branch. Most tumors without *EGFR* mutations (*blue spot*) are clustered with the EGFR-WT branch, although some are clustered with the EGFR-MUT branch. Six tumors carrying K-*ras* mutations (*yellow spot*) are clustered with the EGFR-WT branch and four MET-amplified tumors (*green spot*) are clustered with the EGFR-MUT branch. The EGFR-MUT branch is subdivided into two subgroups (EGFR-MUT-A and EGFR-MUT-B) with distinctive genetic changes. *B*, genetic profiles and patient disease-free survival. Relationship between patients' disease-free survival and genetic classification based on the *EGFR* mutation – related loci. EGFR-WT and EGFR-MUT groups were significantly different ($P = 0.01$).

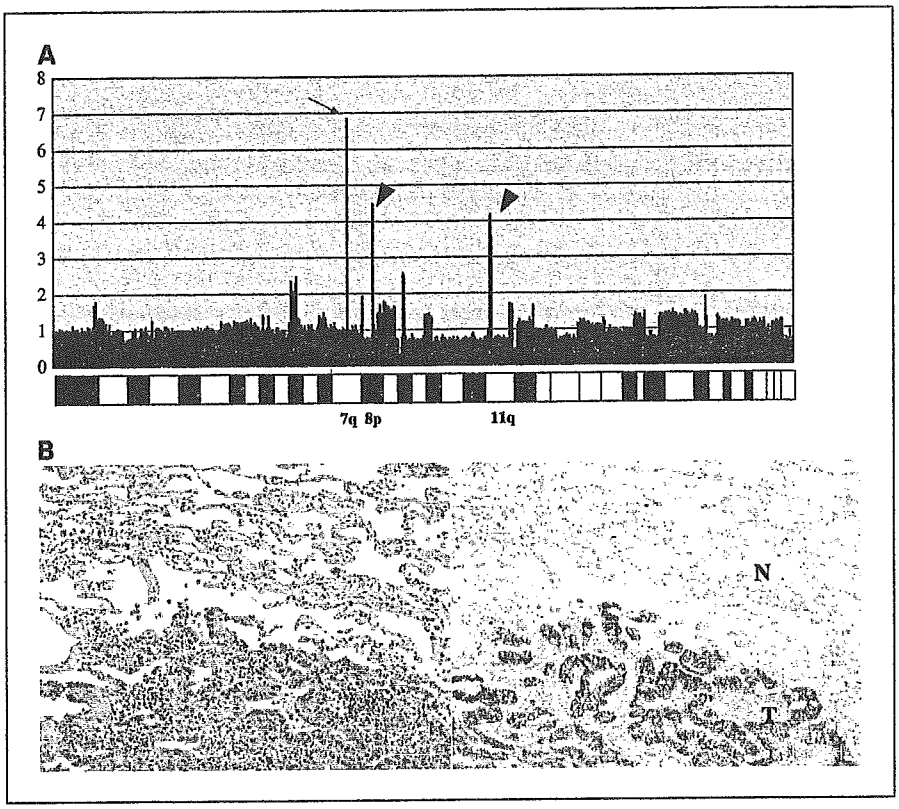

Fig. 3. Amplification and overexpression of MET in lung adenocarcinoma. *A*, a chromosomal copy number alteration profile of a lung adenocarcinoma with an amplification of *MET* locus. Each signal ratio (tumor/normal) of 800 examined loci was plotted from chromosome 1p (*left*) toY (*right*). Each chromosome is represented by underlining boxes as in Fig. 1A. Chromosomal arms (7q, 8p, and 11q) with high-level amplifications were indicated. More than 6-fold amplification of a bacterial artificial chromosome containing the *MET* gene (*7q21*) was detected (*arrow*). This tumor also showed amplifications on 8p and 11q (*arrowheads*). *B*, immunohistochemical analysis of MET protein expression in the same case (×200). Four-micrometer sections of formalin-fixed, paraffin-embedded specimens were stained with H&E (*left*) and an anti-MET monoclonal antibody (*right*). Overexpression of the MET protein in tumor cells (*T*) was observed compared with the surrounding noncancerous lung tissue (*N*). All MET-amplified tumors exhibited prominent membranous and cytoplasmic expression. *Bar*, 200 μm.

55 cases and 36.3% of total cases, respectively) shared many genetic alterations but also had changes unique to each other. This implied that they may be derived from a common precursor and diverge via the acquisition of specific genetic alterations during tumor development. In contrast, the third subclass (cluster B, 15 of 55 cases, 27.3%) showed characteristically fewer genetic alterations than the other two. Although one would expect this group to consist of tumors of an earlier stage, it contained tumors that varied clinically (from stage I to stage III) and there was no significant correlation of histopathologic features with the above classification. Interestingly, this clustering classification is significantly associated with smoking habits, suggesting that the specific carcinogen exposure may affect overall genetic profile of lung cancer. We propose three possibilities for the carcinogenesis process in the third group; these tumors may predominantly acquire (*a*) genetic alterations not covered by our arrays, although they contain most of the known cancer-related genes; (*b*) genetic alterations that do not involve copy number changes, such as balanced chromosomal translocations or microsatellite instability (37, 38); or (*c*) epigenetic alterations such as aberrant methylation of gene promoters, which have been reported to associate with smoking history (39). Further analysis of this group, focusing on the above mechanisms, will provide a more complete view of lung carcinogenesis.

Because the *EGFR* gene is frequently altered in lung adenocarcinoma and its mutation status is correlated to the sensitivity to the specific inhibitor, Gefitinib (8–10), we assumed that the *EGFR* pathway plays important roles in lung cancer and examined whether *EGFR* mutated tumors have any genetic characteristics in nature. We detected the *EGFR* gene mutations at similar frequency as reported (31, 32, 40) and the presence of somatic mutations was significantly associated with never-smoking history as previous studies reported (8–10, 31, 32, 40). We detected K-*ras* mutations relatively less frequent than previously reported (41) but comparatively to other study (42) probably because our analyzed cases contained more female and nonsmokers. We found that *EGFR* mutation and K-*ras* mutation were mutually exclusive as reported (31, 32, 40) and this finding is consistent with the notion that activation of both *EGFR* and K-*ras* stimulates the same downstream pathway (43).

We identified 58 loci whose alterations significantly correlated with the presence of *EGFR* mutations. It is interesting to note that amplification of the *EGFR* gene itself is significantly observed in *EGFR* mutated tumors, indicating that both somatic mutation and amplification of the *EGFR* gene simultaneously occur in part of lung adenocarcinoma. Using these selected loci, we classified the tumors by supervised hierarchical clustering. This classification revealed two groups: one containing only *EGFR* wild-type tumors (EGFR-WT) and the other (EGFR-MUT) containing all *EGFR* mutated and some *EGFR* wild-type tumors. Because the *EGFR* wild-type tumors that were grouped with the EGFR-MUT group shared similar genetic alterations with the *EGFR* mutated tumors, we hypothesized that they may have unknown genetic alterations complementary to *EGFR* activation and subsequently examined loci containing oncogenic receptor–type tyrosine kinases in our arrays. We found that a locus (*7q21*) containing the *MET* gene was amplified in part of these *EGFR* wild-type tumors and immunohistochemically validated overexpression of MET protein in these tumors. MET was shown to be implicated in *ras*-mediated tumorigenicity (44, 45) and activated in many tumors (34–36). Although the number of cases with MET amplification is small in this study, it is tempting to speculate that amplification of the *MET* gene may play a role similar to *EGFR* mutation in lung adenocarcinoma. Recently, somatic alterations of the *MET* gene were detected in lung cancer and pharmacologic inhibitors specific to the MET kinase have been

reported (46–48). Our results also support the idea that the MET oncoprotein is a potent new candidate for therapeutic target in lung adenocarcinoma although there was no somatic mutation in the analyzed exons of our cases. In our cases, there are seven tumors without either *EGFR* mutations or *MET* amplification in the EGFR-MUT group. Somatic mutations in the kinase domain of *ERBB2* were reported in *EGFR* wild-type lung adenocarcinomas (7, 33). Therefore, we searched for *ERBB2* mutations in all 55 cases and found no somatic mutations, suggesting that other oncogenic kinases might be involved in these tumors.

EGFR mutation status could not predict tumor recurrence, which is consistent with a previous report on the insignificant relationship between *EGFR* mutation and patient prognosis (40). However, we found that EGFR-MUT group, which is revealed by genetic classification, showed significantly shorter disease-free survival than EGFR-WT group. Our results imply the possibility that specific combinations of genetic alterations (genetic code) selected by genome-wide analysis could evaluate tumor characteristics and estimation of such codes would be applicable for diagnostic purposes. Our classification also revealed that there are two genetically distinctive subgroups in the *EGFR* mutated lung adenocarcinoma, which were associated with tumor histologic differentiation. Because Gefitinib is one of the most promising molecular target drugs against lung cancer and molecular mechanisms determining its efficacy are still unclear (49), further analysis of a larger cohort is warranted to determine any possible relationship of genetic profiling with sensitivity to chemotherapeutic agents, including tyrosine kinase inhibitors.

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Institutional report - Thoracic general

The new strategy of selective nodal dissection for lung cancer based on segment-specific patterns of nodal spread☆

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Abstract

A new strategy for selective nodal dissection in non-small cell lung cancer (NSCLC) patients according to the segment of primary tumor was explored. Data on 504 patients with NSCLC of less than 5 cm, histologically revealed to be N2 disease after thoracotomy, were analyzed. In right upper lobe (RUL) tumor, when the pretracheal node was negative, the incidence of subcarinal involvement was 3.8%. In lower lobe tumor, superior segment (RLL-Superior and LLL-Superior) tumor showed a significantly higher incidence of superior mediastinal involvement than basal segment (RLL-Basal and LLL-Basal) tumor (right, $P=0.0036$; left, $P=0.0499$). When the subcarinal node was negative, the incidence of superior mediastinal metastasis in RLL-basal and LLL-Basal tumor was 11% and 8%, respectively. In left upper lobe tumor, superior segment (LUL-Superior) tumor showed a significantly lower incidence of subcarinal involvement than lingular segment (LUL-Lingular) tumor ($P=0.0381$). When aortic nodes were negative in LUL-Superior tumor, the incidence of subcarinal metastasis was 6%. Collectively, in RUL and LUL-Superior tumors, subcarinal dissection may be unnecessary if superior mediastinal node is negative. In RLL-Superior and LLL-Superior tumors, extensive dissection is required. In RLL-Basal and LLL-Basal tumors, superior mediastinal dissection may be unnecessary if subcarinal node is negative.

Keywords: Selective nodal dissection; N2; Systematic nodal dissection; Non-small cell lung cancer

1. Introduction

Since Cahan (1960) [1] reported the first 48 cases that successfully underwent lobectomy with regional lymph node dissection, which was called radical lobectomy, this procedure has been a standard surgery for lung cancer. In 1978, Naruke [2] suggested an anatomical map in which the lymph node stations were numbered, and since then this map has been used for nodal dissection. With this map, extensive nodal dissection including the superior and inferior mediastinum has been universally performed in lung cancer surgery. This technique, termed systematic nodal dissection (SND) remains an important component of the investigative and therapeutic process in all patients undergoing thoracotomy for lung cancer.

However, as the number of early-detected lung cancers is increasing with the recent development of the CT scanner, the extent of nodal dissection for lung cancer should be tailored to each case. That is, more selective dissection should be undertaken especially for early cancer by considering the tumor location-specific lymphatic pathway, simply because nodal involvement is not so extensive in many cases. In this study, a new strategy for selective nodal dissection in non-small cell lung cancer (NSCLC) patients based on segment-specific patterns of nodal spread was explored.

2. Materials and methods

2.1. Patients

Data on 504 patients with NSCLC less than 5 cm, histologically revealed to be N2 disease between January 1977 and October 2003, were analyzed. Tumors invading the other lobe were excluded. All patients underwent at least lobectomy with hilar and mediastinal lymphadenectomy. The correlation between the segment of the tumor location and the involved hilar/mediastinal nodes were investigated in each case.

2.2. Surgical procedure

Pulmonary resection and SND were performed through posterolateral thoracotomy. At thoracotomy the diagnosis was confirmed by frozen-section analysis, if histological confirmation was not available preoperatively. Systematic nodal dissection, including the superior to inferior mediastinum, was then performed after pulmonary resection. In left thoracotomy, upper mediastinal dissection indicated aortic (#5, 6) and tracheobronchial (#4) node dissection.

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Table 1
Patient characteristics in pathological N2 non-small cell lung cancer patients less than 5 cm in size

Number of patients	504
Histological type	
Adenocarcinoma	367 (72.8%)
Squamous cell carcinoma	99 (19.6%)
Large cell carcinoma	27 (5.4%)
Adenosquamous carcinoma	9 (1.8%)
Others	2 (0.4%)
Tumor location	
Right	303
upper lobe	183
middle lobe	25
lower lobe	95
Left	201
upper lobe	140
lower lobe	61

2.3. Patient characteristics

Patient characteristics are shown in Table 1. The tumor cell types were adenocarcinoma in 367 (72.8%), squamous cell carcinoma in 99 (19.6%), large cell carcinoma in 27 (5.4%) and adenosquamous cell carcinoma in 9 (1.8%). Right thoracotomy was performed in 303 patients and left in 201. The lobe of origin was the right upper lobe (RUL) in 183 patients, right middle lobe (RML) in 25, right lower lobe (RLL) in 95 in 41 of whom it was the superior segment, left upper lobe (LUL) in 140 in 122 of whom it was the superior segment, and left lower lobe (LLL) in 61 in 23 of whom it was the superior segment.

2.4. Statistical analysis

The statistical significance of differences was determined using the chi-square test for independence. Relative risk and 95% confidence intervals were calculated. Values of P less than 0.05 were considered to be statistically significant.

3. Results

3.1. RUL tumor

The incidence of subcarinal involvement in RUL tumor was 18% (33/183). However, when the pretracheal lymph node (#3) was negative, the incidence was only 3.8% (7/183). There were no significant differences in patterns of lymphatic pathway between the apical, posterior and anterior segments within the RUL.

Table 2
The incidence of upper mediastinal metastasis in superior and basal segment tumor of the lower lobe

Side	Location of the primary tumor	No. of patients	Metastasis to the superior mediastinal nodes		
			No.	%	P value
Right	Superior segment	41	26	63	0.0036
	Basal segment	54	18	33*	
Left	Superior segment	23	15	65	0.0499
	Basal segment	38	15	39**	

* When subcarinal lymph node (#7) was negative, the incidence of superior mediastinal (#1–4) metastasis was 9% (5/54).
** When subcarinal lymph node (#7) was negative, the incidence of superior mediastinal (#4, 5, 6) metastasis was 8% (3/38).

3.2. RML tumor

The incidence of superior mediastinal (#1–4) and subcarinal (#7) involvement was 52% (13/25) and 72% (16/25), respectively. The incidence of lower mediastinal involvement was 8% (2/25). There were no significant differences in patterns of lymphatic pathway between lateral and medial segment within the RML.

3.3. RLL and LLL tumor

Among all of the segments in the lower lobe, 5 segments in the right lung and 4 in the left, there were significant differences in patterns of lymphatic pathway between the superior and basal segments on both sides, as shown in Table 2. The incidence of superior mediastinal involvement in superior segment tumor (right 65%, 26/41; left 65%, 15/23) was higher than that in basal segment tumor (right 33%, 18/54; left 39%, 15/38), with significant differences (right, $P=0.0036$; left, $P=0.0499$). When the subcarinal lymph node (#7) was negative, the incidence of superior mediastinal metastasis in RLL-basal and LLL-Basal segment tumor was 9% (5/54) and 8% (3/38), respectively.

3.4. LUL tumor

There were significant differences in patterns of lymphatic pathway between the superior and lingular segments within the LUL, as shown in Table 3. Superior segment tumor showed a significantly lower incidence of subcarinal involvement (14%, 17/122) than lingular segment tumor (33%, 6/18) ($P=0.0381$). When aortic lymph nodes (#5, 6) were negative in superior segment tumor, the incidence of subcarinal metastasis was 6% (7/122).

Collectively, the following eight segments, four in each side lung, with specific lymphatic pathways were identified: RUL ($n=183$), RML ($n=25$), superior segment of the RLL (RLL-Superior, $n=41$), basal segment of the RLL (RLL-Basal, $n=54$), superior segment of the LUL (LUL-Superior, $n=122$), lingular segment of the LUL (LUL-Lingular, $n=18$), superior segment of the LLL (LLL-Superior, $n=23$) and basal segment of the LLL (LLL-Basal, $n=38$). Based on the abovementioned patterns of nodal spread, the proper strategy for the selective lymph node dissection of each segment is shown in Table 4.

4. Discussion

The pathological nodal status in lung cancer patients is not always the same as that predicted by pre-operative investigations. For TNM classification, CT scan has been used in the clinical diagnosis of nodal status, however, the sensitivity of CT scan for the N factor is reported about 64 to 77% [3]. Since a high incidence of false-negative nodes on CT scan has been reported [4], systematic nodal dissection (SND), which means extensive mediastinal dissection including superior to inferior mediastinum, has been per-

Table 3
The incidence of superior mediastinal and subcarinal metastasis in superior and lingular segment tumor of the left upper lobe

Location of the primary tumor in the left upper lobe	No. of patients	Metastasis to the superior mediastinal nodes (#4,5,6)			Metastasis to the subcarinal node (#7)		
		No.	%	P value	No.	%	P value
Superior segment	122	118	97	NS	17	14*	0.0381
Lingular segment	18	13	72		6	33	

* When aortic nodes (#5, 6) were negative, the incidence of subcarinal metastasis was 6% (7/122).

formed for lung cancer patients undergoing thoracotomy. Pathological evaluation of nodal involvement at the mediastinal and hilar levels is essential for detailed assessment of the disease extent.

Graham and associates [5] suggested that SND could disclose unexpected N2 disease, irrespective of cell type, the size, location and lobe of origin of the primary tumor, and whether prior mediastinoscopy had been performed. Keller and associates [6] suggested that cure rates could be improved by SND. Therefore, SND has been accepted as an important component of the investigative and therapeutic process in NSCLC patients.

With the development of the CT scanner and the increased incidence of lung cancer, the number of early-stage lung cancer is rising. The incidence of small-sized lung cancer among resected primary lung cancers in recent years has exceeded 20% in Japan [4,7]. As the number of early-detected lung cancers is increasing, a new therapeutic strategy for nodal dissection is required. Proposals of limited surgery for lung cancer have been undertaken in some previous reports [8–10].

There are two methods of limited surgery, one is lung parenchyma-preserving surgery and the other is limited nodal dissection. Regarding the lung parenchyma-preserving surgery, Lung Cancer Study Group (LCSG) [11] reported the results of a randomized trial of lobectomy versus limited resection for T1N0 NSCLC. They observed a 75% increase in recurrence and a 50% increase in cancer death in the patients undergoing segmentectomy or wedge resection, compared to those in the patients who underwent lobectomy. It is difficult to select candidate patients for limited resection, because cT1N0 lung cancer patients show nodal disease with a 15 to 25% incidence [4,7].

As for limited lymph node dissection, Riquet and associates [12] reported that lung cancer metastasizes so easily to the mediastinum that selection of the patients for limited surgery should be discussed carefully. Some previous reports have described the appropriateness of selective nodal dissection based on the lobe-specific extent of nodal spread [13–15]. In this study, we evaluated more meticulous data of the lymphatic pathway in not only T1 but also T2 tumors, to collect as much data as possible, and proposed a method of limited dissection from these results as shown in Table 4. The strategy of lymph node dissection should be changed from extensive dissection to selective dissection especially in early stage cancer or poor risk patients, because selective dissection will be able to reduce postoperative morbidity, such as bronchopleural fistula, chylothorax or recurrent nerve palsy. The establishment of a universally accepted method of selective nodal dissection for lung cancer would be indispensable.

5. Conclusions

Based on the patterns of nodal spread, a proper strategy for selective lymph node dissection of each segment was proposed as shown in Table 4. In RUL and LUL-Superior tumors, subcarinal dissection may be unnecessary if the superior mediastinal node is negative on frozen section. In RML and LUL-Lingular tumors, superior mediastinal and subcarinal dissection is necessary. In RLL-Superior and LLL-Superior tumors, extensive systematic dissection is routinely required. In RLL-Basal and LLL-Basal tumors, superior mediastinal dissection may be unnecessary if the subcarinal node is negative on frozen section.

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Table 4
The strategy of selective nodal dissection based on segment-specific patterns of nodal spread

	Location of the main tumor			
	RUL LUL-Superior	RML LUL-Lingular	RLL-Superior LLL-Superior	RLL-Basal LLL-Basal
Superior mediastinal nodes *3	⊙	⊙	⊙	O*2
Inferior mediastinal nodes				
Subcarinal node (#7)	O*1	⊙	⊙	⊙
Paraesophageal (#8) and pulmonary ligament (#9) nodes	×	×	⊙	⊙

⊙ dissection is advisable, O dissection is not always necessary, × dissection will be unnecessary.
*1: dissection may be unnecessary if pretracheal node (#3) is negative on frozen section.
*2: dissection may be unnecessary if subcarinal node (#7) is negative on frozen section.
*3: #1–4 for the right side, and #4–6 for the left.

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Appendix. Conference discussion

Dr. P. van Schil *(Edegem, Belgium)*: I think this is especially important as within the ESTS we are working on guidelines for peroperative mediastinal staging.

I have two questions for you. Did you observe any difference with the previous studies performed by Japanaese surgeons; for example, the classical studies by Naruke? Secondly, you state that you do segment-specific nodal dissection. On the other hand, you consider the upper lobe and the middle lobe just as 1 segment, but, in fact, anatomically there are 3 segments in the upper lobe and 2 in the middle lobe, or doesn't it matter for those 2 lobes?

Dr. Watanabe: Let me anaswer the second question first. I checked all of the segments in the right upper lobe and middle lobe, that is, the apical, posterior and anterior segment in the upper lobe, and the lateral and medial segment in the middle lobe. But there are no specific pathways among those segments, so I divided just the right upper lobe and the right middle lobe.

I'm sorry, what was the first question?

Dr. van Schil: Did you observe any differences with previous studies performed by the Japanese surgeos; for example, the studies by Naruke?

Dr. Watanabe: Unfortunately, no.

Dr. D. Branscheid *(Grosshandsdorff, Germany)*: Do you think that there are sometimes reasons why the lymph nodes are flowing more in a certain direction and all of a sudden there are 2 or 3 and the flow is to another side? Did you check if they have had tuberculosis or other infections? Have those lymph nodes been enlarged or have they been normal on the CR scan? Can you give us something about that?

Dr. Watanabe: I didn't check all of your suggestions. We basicaly do surgery only for clinical N0 and N1 patients.

Dr. Branscheid: Let me just ask another question. When the situation is like that, isn't it just an argument to do a complete dissectin and not say, okay, this node is not involved, therefore I probably do not need to take the next one? This is the consequence that I take out of your presentation. Is that wrong, or do you see it like that also a little bit?

Dr. Watanabe: Your question is why we are exploring the selective nodal dissection, why we don't do systematic nodal dissection?

Dr. Branscheid: No. Is the consequence to do a complete dissection? Is this your consequence out of that?

Dr. Watanabe: No. Recently, with the development of the CT scanner in Japan, we are getting a large number of early lung cancers. Basically we need to do systematic nodal dissection for lung cancer patients, but for early stage lung cancer we don't think systematic dissection is required for all of the tumors. This is the reason why we started this study. But the candidate in this study was the patients who underwent systematic dissection. So basically we think that systematic dissection is very important for lung cancer.

Dr. S. Elia *(Rome, Italy)*: You said that in 8 of 12 cases, actually in 66%, you would advice lymph node dissection. So you leave only 33% of lymph nodes that are actually doubtful. Would you feel safe in not performing complete lymphadenectomy in these patients? What is your conclusion?

Dr. Watanabe: I just took only pathological N2 patients. But among all patients, I mean N0, N1 and N2, if we included those patients, the incidence is going down, and I think the incidence will be one-third of this figure. So I think if the incidence is less thatn 10%, the incidence among all the lung cancer patients will be a few percent.

Dr. A. Turna *(Istanbul, Turkey)*: Could you tell us how many of your patients had mediastinoscopy before resection? Did you perform mediastinoscopy in these patients?

Dr. Watanabe: Very few: Basically we do mediastinoscopy for clinical N2 patients, and they are actually N2 on the mediastinoscopy. So we excluded those patients. In this study, the number of patients who underwent mediastinoscopy is very few.

Dr. B. Passlick *(Freiburg, Germany)*: What is your strategy for the future? Will you leave the upper mediastinal nodes in place if you have a right lower lobe tumor and a negative forzen section on the No. 7 lymph nodes?

Dr. Watanabe: We are now studying that kind of selective dissection. If the hilar lymph node and the No. 7 lymph node are negative in basal segment tumor, we can omit the upper mediastinal dissection. But now we are conducting only for poor-risk patients or very early lung cancer patients.

Meeting Summary of the 12th International Conference on Screening for Lung Cancer: Nara, Japan, April 2005

Kenji Eguchi, MD, PhD, and Claudia Henschke, MD, PhD†*

(*J Thorac Oncol.* 2006;1: 190–197)

The 12th International Conference on Screening for Lung Cancer, hosted by the Screening Committee of the Japan Lung Cancer Society, was held in Nara, Japan on April 8–10, 2005. The International Early Lung Cancer Action Program (I-ELCAP) has conducted regular conferences semiannually both at Weil Cornell Medical College in New York, NY and at other I-ELCAP sites in the world. This 12th conference was a landmark of I-ELCAP activity for 6 years. Low-dose computed tomographic (LD-CT) scan was experimentally introduced for lung cancer screening in both Japan and the United States in 1993. Commemorating the pioneering work on LD-CT screening for lung cancer for a decade by both the ELCAP and the Japanese group, this I-ELCAP conference was hosted by the Screening Committee of the Japan Lung Cancer Society under the auspices of the Japan Lung Cancer Society, the Japanese Respiratory Society, the Japanese Society for Respiratory Surgery, the American Cancer Society, and the International Association for Studies of Lung Cancer (IASLC). The broadest mission of these conferences is the collective pursuit of avant-garde understanding of the issues surrounding screening for lung cancer, the broadest subissues being early diagnosis and early intervention. Any given conference focuses on issues that are of particular interest at the time. As always, the conference provides an update of research on and practice of screening for lung cancer, including updates of I-ELCAP protocols and results of research. For the conference, more than 180 attendees from eight countries, including non-members of the I-ELCAP, gathered in Nara, one of the ancient capitals of Japan in the seventh to eighth century (Figure 1).

The 10th and 11th Conferences focused on the results on the diagnostic performance of the I-ELCAP protocol for CT screening and on alternatives to resection in early intervention. These conferences also focused on the discussion between individuals seeking screening and their physicians such as the potential benefit of a single round of screening. However, it requires the likelihood that early intervention could cure such a cancer, and that the patient would avoid death from another cause for a decade or another specified period. The 12th International Conference aimed to summarize the evaluation by I-ELCAP and Japanese groups as to the benefit of CT screening for lung cancer.

CONSENSUS STATEMENT

This Nara conference addressed the two broad missions of these conferences: advancement policy (relevant research on early diagnosis of lung cancer) and translation of up-to-date findings into guidelines for practice based on the accumulated evidence from the I-ELCAP consortium. Experiences with screening performed by individual institutions were presented for the Japan Association against Lung Cancer (ALCA), Hitachi's employee screening program, and the Mayo Clinic screening program. The Japanese Lung Cancer Screening Study (JLCSS) group reported on its nationally based study comparing 46,733 people who had at least one CT screening test with 91,970 people who had at least one chest radiographic screening. I-ELCAP presented its data for more than 28,000 people who have had more than 50,000 screenings. Thus, this conference reviewed the largest studies ever conducted with CT screening for lung cancer.

The final results of the JLCSS will be reported in 2007, but a 2005 interim report indicated that it will show a reduction in deaths from lung cancer as a result of screening. The I-ELCAP presented its approach to screening research, development of the regimen and assessing its benefit, and distinguishing between the screening's diagnostic and prognostic implications. Diagnostically, the concern centers on how early the diagnosis of lung cancer can be achieved while minimizing work-up, including biopsies. Relative to prognosis, the issue is the preventability of death from lung cancer by early intervention based on early diagnosis. In the I-ELCAP experience, more than 80% of diagnoses have been achieved at stage I, with 90% of the biopsies resulting in a diagnosis of malignancy. Critical in the regimen is the identification of growth consistent with malignancy. The I-ELCAP also presented results pertaining to prognosis. These confirm that screen-diagnosed lung cancers presenting as solid nodules lead to death if not treated, whereas some cases of adenocarcinoma presenting as a non-solid nodule may be so slow-growing as to be unlikely to be fatal for a short time if not treated. It is estimated that more than 80% of deaths from lung cancer could be prevented by early intervention under CT screening. The I-ELCAP also discussed competing

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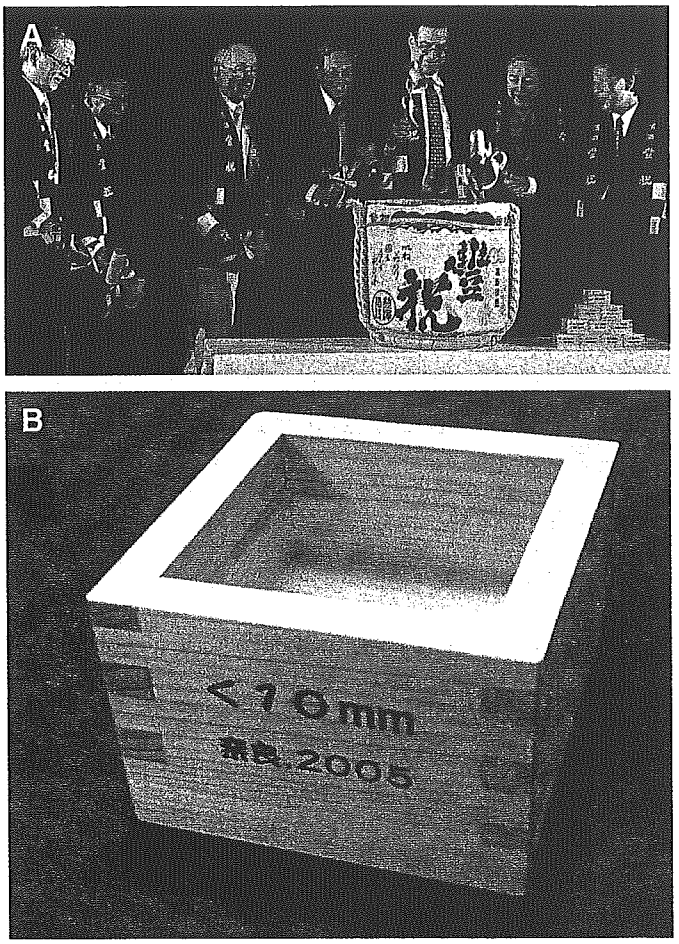

FIGURE 1. (A) "Kagamibiraki," Opening a Japanese sake barrel head at the reception (the Garden of Nara-Ken New Public Hall). (B) A wooden cup for Japanese sake printing <10 mm. It is challenging to detect nodules <10 mm in diameter, that is, "curable lung cancer."

causes of death in the highest-risk subcohort of people aged 60 to 75 years with at least 30 to 80 pack-years of smoking. It found that the 5- and 10-year rates of death from causes other than lung cancer (conditional on not dying from lung cancer) were low, 3% and 7%, respectively.

I-ELCAP surgical and radiotherapy results were presented together with those of Japanese studies on limited resection, transbronchoscopic brachytherapy, percutaneous microwave and radiofrequency ablation, cryosurgery, heavy-particle radiation therapy, and stereotactic radiation therapy. Pathologic and molecular features of early cancer were also presented. These results provided the foundation of new treatment trials on screen-diagnosed lung cancers.

Three workshops were held. One of these focused on the methodological advances and safety of CT-guided fine-needle aspiration biopsy and issues of follow-up in biopsy-negative cases central to a regimen of screening. Another workshop focused on multicenter treatment trials, such as different protocols depending on CT features of nodules and the critical issues to be studied. The third workshop focused on computer-aided detection and characterization to deal with the increasing number of images per person that can now be produced by a single CT scan.

ORAL PRESENTATIONS: CURRENT STATUS AND EXPLORATORY STUDIES OF LUNG CANCER SCREENING

Keiichi Nagao, MD, (Safety and Health Organization, Chiba University, Japan) discussed practical problems in the application of LD-CT screening in Japan. In Japan, more than 100,000 subjects received LD-CT scans as practice-based opportunistic screening annually. These screenings are partly supported by local governments or by insurance in Japan. He addressed five major issues: reducing cost to the individual for screening, increasing subsidies from local governments, expanding the training of specialists for LD-CT reading, application of computer-aided diagnosis (CAD), and qualification of hospitals that had specialists to perform further examinations and treatment for small lung cancers detected in screening.

Tomotaka Sobue, MD, (Research Center for Cancer Prevention and Screening, National Cancer Center Tokyo) presented the historical background of lung cancer screening in Japan. The lung cancer screening system has been developed based on well-established mass screening system for tuberculosis, which had been in operation since 1951 and continued in the 1970s and 1980s. In 1987, lung cancer screening using chest radiographs (and sputum cytology for high-risk populations) for people aged 40 years or older was introduced as a national policy under the Health Services Law for Aged. This decision was not based on direct evidence of a benefit from lung cancer screening in terms of a reduction of mortality. Since 1992, six case-controlled studies from different large groups of Japanese have been published. Although they were retrospectively analyzed, all studies indicated beneficial effects, and four studies showed statistically significant mortality reductions of lung cancer. There is no evidence that the difference between the trend of incidence and mortality of lung cancer has been widened in Japan, and the facts indicate that lung cancer screening in Japan has not been effective at a national level. However, in the areas in which these studies were conducted, lung cancer mortality among women aged 50 to 74 years has been decreasing. Lung cancer screening using chest radiographs and sputum cytology is effective only when it is applied with high quality assurance, and many of the systems in Japan have not reached this level.

Hironobu Ohmatsu, MD, (National Cancer Center East Hospital, Japan) presented the 10 year-experience of LD-CT screening in the ALCA project in Tokyo. ALCA is a for-profit organization to screen dues-paying members (smokers aged 40 years or older) for lung cancer that was established in 1975 by Shigeto Ikeda, MD, who had was the first to develop flexible bronchofiberscopy. In 1993, LD-CT was introduced as a screening device at ALCA. Multi-detector CT (four rows) has been used since 2002. Using 15 mAs of radiation exposure, the data were acquired with 2-mm collimation and 10-mm reconstruction. From 1993 to 2004, a total of 18,331

screenings have been performed, and 76 lung cancers (0.44%) have been detected. Compared with historical data, the ALCA detection rate was 3 times higher than that of screening with a chest radiograph. Ninety two percent of the cancers were peripheral type, and 62% were adenocarcinoma. Mean tumor size was 17 mm, and 80% were stage I (74% stage IA). The 5-year survival rate was 80.4%. In terms of stage shift in patients with invasive adenocarcinoma, results showed statistically significant shifts to early stage (stage I) among the detected lung cancers during long-term repeated LD-CT screening for more than 10 years. In other histology, no statistically significant stage shift was seen.

James Jett, MD, (Mayo Clinic, USA) summarized the final results of the Mayo trial on LD-CT screening. A total of 1520 high-risk participants aged 50 years or older were enrolled and received five scans for 4 years. Non-calcified nodules were detected in 51% of participants at the baseline screening and in 73.6% after the fifth annual screening. Of the non-calcified nodules, 61% were 4 mm or smaller in diameter. Lung cancer was detected in 66 participants with 68 lesions. Sixty-five percent of prevalent and 62% of incidence cancers were stage IA. Ten participants (18%) underwent surgical procedures for benign nodules that had been detected by screening. Screening high-risk individuals with LD-CT detected early-stage lung cancer but also detected a large number of non-calcified nodules that required periodic follow-up scans.

Toru Nakagawa, MD, (Hitachi Health Care Center, Japan) presented the 6-year experience on LD-CT screening at the Hitachi Electric Co. Ltd. Among 12,645 individuals, 60 lung cancers were surgically diagnosed (0.47%). Of these tumors, 90% were pathologically stage I. Annual repeat screening was performed for 24,889 subjects, and 22 lung cancers were detected. The detection rate was 0.09%, and all were pathologically stage I. Dr. Nakagawa stressed that repeat screening for the same cohort would be a powerful tool with which to study the efficacy of a screening method like LD-CT (Figure 2).

There were two other presentations on screening-related studies: one was biomarker analysis of the aggressiveness of CT screen-diagnosed tumors by Luis Montuenga, MD, (Clinica Universitaria de Navara, Spain); the other was competing causes of death in a lung cancer screening cohort by Rowena Yip, MPH (Weil Medical College of Cornell University, New York, NY).

ORAL PRESENTATIONS: TWO LARGE COHORT STUDIES FOR LD-CT SCREENING

Tomio Nakayama, MD, PhD, (Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan) presented an interim analysis of a nationally based cohort study on LD-CT screening from the JLCSS group. This is a non-concurrent cohort study with a control arm of participants receiving a chest radiograph. Eligible subjects were aged more than 40 years and were never screened by LD-CT, excluding patients who had been diagnosed with or were thought to have lung cancer. The subjects who received LD-CT or a chest radiograph as screening from 1995 to 2002 were enrolled. The

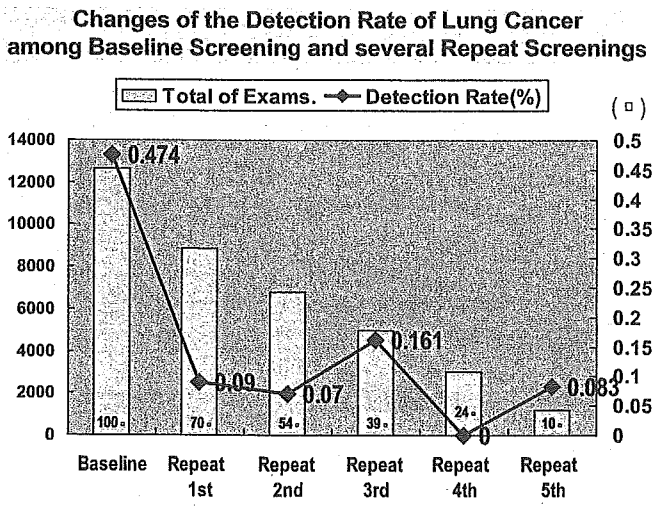

FIGURE 2. Toru Nakagawa, MD (Hitachi Health Care Center). Changes of the detection rate of lung cancer at the baseline screening and repeat screenings.

study began in 2001, and the final analysis will be performed in 2007. The sample size is an estimated 40,000 subjects for the LD-CT cohort and 80,000 subjects for the usual chest radiograph screening cohort to detect a 30% reduction of lung cancer mortality where the effect of usual screening is 30% and the pure effect of LD-CT screening is 51% ($\alpha = 0.05$, $\beta = 0.2$ two-sided test). The age of both groups is 40 to 74 years old, and follow-up period is 5 years. From nine screening groups in different districts of Japan, a total of 46,733 subjects were enrolled as the LD-CT group and 91,970 subjects as a control group. In six screening groups, the subjects were enrolled as a community-based screening and included a large number of female nonsmokers (Table 1). The detection rate of lung cancer in the LD-CT group was 0.69% in men and 0.64% in women: 3.6 and 9 times higher than those of the chest radiograph group, respectively. In repeated screenings, detection rates in the LD-CT group decreased to 0.08%. The rate of stage IA screen-detected lung cancers was 83% and 91% in baseline and repeated screenings, respectively. Currently, 850 deaths from all causes have been identified in the LD-CT screening group versus 3480 deaths in chest radiograph group, compared with 85 lung cancer deaths in the former group and 336 deaths in the latter group. In preliminary analysis, the discrepancy between overall mortality and lung cancer mortality of the cohort became gradually larger in men; however, it was not statistically significant (Figures 3).

TABLE 1. Performance of Lung Cancer Screening in JLCSS

	CT screening cohort		Usual screening cohort	
	Male	Female	Male	Female
Detected lung cancer (n)	216	112	64[a]	36[a]
Detection rate (%)	0.73	0.65	0.19*	0.07†

From the presentation by Tomio Nakayama, MD. CT, computed tomography; WHO, World Health Organization. [a] Lung cancer cases of the usual screening cohort are not applicable. *$P < 0.05$. †$P < 0.001$.

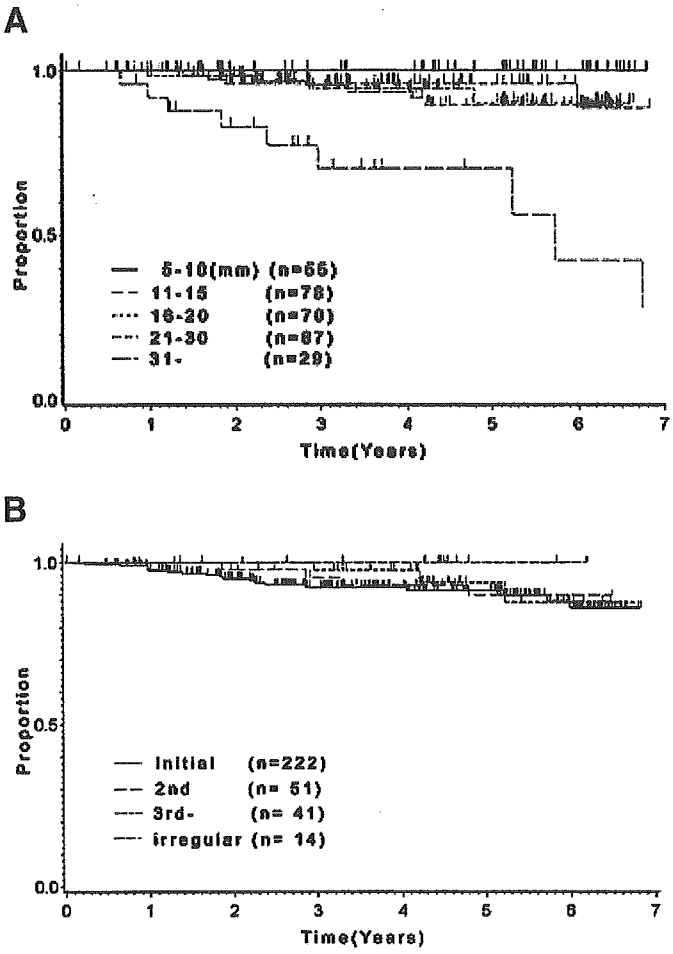

FIGURE 3. Tomio Nakayama, MD. *A*, Survival of patients with LD-CT–screened lung cancers by size (JLCSS). *B*, Survival of patients with LD-CT–screened lung cancers by cycles of screening (JLCSS).

Data with the lung cancer-specific mortality rates for each cohort, together with all caused mortality, will be presented as a final analysis in 2007. As a subset analysis, a significant difference in survival among subjects with LD-CT–detected lung cancer was found depending on the tumor size. Subjects with tumors smaller than 1 cm had better survival than others, and subjects with tumors larger than 3 cm had the lowest survival rate.

Claudia Henschke, MD, PhD, (Weil Medical College, Cornell University) reported the summarized data of the 6-year I-ELCAP project.

The ELCAP approach consists with two parts. Part A is a diagnostic mission, whereas Part B is a prognostic mission. In Part A, lung cancer distribution, such as stage, size, etc., will be clarified using the data obtained from baseline and annual repeat screenings. In Part B, the fatality rates of patients with screen-detected lung cancer, treated or non-treated/delayed treatment will be clarified specific to stage and size.

The mission of I-ELCAP is to advance policy-relevant research on the early diagnosis of lung cancer and to foster translation of up-to-date evidence into guidelines for practice. The advantage of the I-ELCAP research approach is multifaceted. It should further state-of-the-art screening research and clinical practice, it should provide for continual updating in light of advancing knowledge, and result in incorporation of technologic advances as they develop. I-ELCAP has accumulated 28,689 baseline CT screenings and 20,706 annual repeat CT screenings at 38 institutions throughout the world. Under the systematic protocol, 253 lung cancers and 31 lung caners have been diagnosed at the baseline screening and annual repeat screening, respectively.

The median diameter of screen-diagnosed lung cancer was 15 and 8 mm in baseline and repeat screening, respectively. More than 80% of screen-detected lung cancers were stage I. A diagnostic performance biopsy proved malignancy in more than 90% of patients. Non-solid type tumors showed no size-stage relationship, whereas solid and semi-solid type tumors did. I-ELCAP revises the protocol considering these results and continues to accumulate new subjects and follow-up.

Curability can be calculated as fatality rate (FR) of untreated minus FR of treated divided by FR of untreated. In x-ray screening, the curability of stage I x-ray screen-diagnosed cancers is estimated to be 67%. According to the SEER database, curability of stage I lung cancers is estimated to be 71% in tumors 15 mm or smaller in diameter and 67% in those 16 to 25 mm in diameter. Curability of screen-detected stage I lung cancer in CT screening is estimated to be 97% if the tumor is a solid nodular type. The percentage of deaths that can be prevented by CT screening is estimated to be 81% (95% CI, 75–87%), whereas deaths prevented by chest x-ray screening is estimated to be 20% and, for those receiving normal care in America, is estimated to be only 5%, according to the 2005 report from the American Cancer Society.

Using the results of I-ELCAP, one can estimate a risk assessment model and the values for decision making regarding CT screening. For instance, a 55-year-old man who smokes more than 30 pack-years has a probability of detection of stage I lung cancer by CT screening of approximately 80%.

General discussion focused on estimation of overdiagnosis as 10% in LD-CT screening, especially involving localized pure ground-glass opacity or non-solid nodules in female nonsmokers, and the necessity of risk-benefit assessment of LD-CT screening. In the case of lung cancer showing solid or part-solid nodules on CT images, the ratio of overdiagnosis may be small. Additional data on the natural course of non-solid nodules should be accumulated.

ORAL PRESENTATIONS: EARLY DIAGNOSIS

Masahiro Kaneko, MD, (National Cancer Center Hospital, Tokyo, Japan), reviewed the history of CT and discussed future progress of CT equipment and diagnostic systems for screening. He stressed rapid advance in software development of CAD and characterization in the field of LD-CT screening. Screening systems applicable to both peripheral and hilar lesions using rapidly reconstructed LD-CT images will be desirable. Less invasive and more accurate

diagnostic intervention for LD-CT–detected nodules should be developed. Virtual bronchoscopy and three-dimensional reconstructed CT images will be potentially useful in performing accurate bronchoscopic biopsy of small peripheral lesions detected by using LD-CT screening, Progress in automated diagnostic support using CAD is mandatory for LD-CT screening.

Takashi Terauchi, MD, (Research Center for Cancer Prevention and Screening, National Cancer Center Hospital, Tokyo, Japan) discussed difficulties in using 2-[^{18}F]fluro-2-deoxy-D-glucose (FDG)-positron emission tomography (FDG-PET) scan for cancer screening. In Japan, more than 60 groups introduced FDG-PET as their daily practice-based opportunistic screening. FDG-PET is a convenient tool for whole-body screening to detect malignancy, but its accuracy is influenced by many factors, such as the size and biological behavior of tumor and the diabetic state of the subject. FDG-PET is not currently justified as a tool of cancer screening. Dr. Terauchi commented that the detection rate of cancer was up to 5% in his preliminary whole-body screening project for 3000 subjects 50 years of age or older, conducted with the approval of the institutional review board of the National Cancer Center in Tokyo. He will continue with follow-up on the same cohort for 10 years with multimodality repeated screening protocol.

Masami Sato, MD, (Miyagi Cancer Center, Japan), presented the results of population-based screening with chest radiograph and sputum cytology, which was conducted in the Miyagi prefecture in the 1990s. They applied an improved method for staining sputum samples from high-risk groups. He stressed the importance of sputum cytology for high-risk groups because the rate of Japanese smokers is still high: 42% of men and 10% of women for the overall population, and 56% and 20%, respectively, in the younger generation. However, there has been a decrease in the number of hilar-type lung cancers. He added that overdiagnosis of occult hilar-type lung cancers was very rare, according the long-term follow-up data from this group.

David Carbone, MD, PhD, (Vanderbilt University) discussed the identification and application of molecular signatures to the diagnosis and treatment for lung cancer. Using matrix-assisted laser desorption/ionization mass spectroscopy, tumor-specific proteins were characterized with small tumor samples. In his proteomics study, 57 proteins were characterized for the discrimination of malignant phenotypes with a sensitivity of 66% and specificity of 99%. Proteomics show useful information as response and prognostic indicators for patients with lung cancer. Studies on the relationship between proteomics and response/resistance to molecular-targeted drugs such as epidermal growth factor receptor and thyrosine kinase inhibitors are advancing. Proteomic patterns obtained directly from serum of nanogram amounts of fresh frozen human lung tumor tissue may allow the classification and prediction of histological groups, as well as nodal involvement and survival in resected non-small cell lung cancer. He displayed a map of whole-body protein analysis showing organ-specific patterns of protein distribution with array-like display of peaks of mass spectroscopy.

Kiyoshi Yanagisawa, MD, PhD, (Nagoya University, Japan) presented protein expression profiles in non-small cell lung cancer, on which he is working with Dr. David Carbone. They were able to obtain more than 2600 mass spectroscopy peaks from histologically selected regions of single frozen sections from 174 resected human non-small cell lung cancer and 27 normal lung tissues. They found 15 protein patterns as prognostic indicators of non-small cell lung cancer. In preliminary studies, proteomics has been shown to discriminate malignant from benign lesions using serum samples. Currently, they have identified dozens of molecular marker proteins of interest using reverse-phase high liquid chromatography followed by sequencing of peptides with an liquid chromatography tandem mass spectroscopy instrument. With further progress of proteomics research, Dr. Yanagisawa mentioned the possibility of identifying each target protein and characterizing the risk factors for specific subgroups and applying early detection of lung cancer in each molecularly characterized subgroup.

SURGICAL TREATMENT FOR SMALL LUNG CANCER

Kenji Suzuki, MD, (National Cancer Center Hospital, Tokyo) presented a prospective study for peripheral lung cancers 2 cm or smaller in diameter. His group chose a surgical procedure depending on findings of thin-section high-resolution (TSHR) CT images. The CT findings were classified as non-solid, part-solid, semi-consolidation, and solid depending on the ratio of the area of ground-glass opacity lesions on TSHR CT images. The end point of this study was local recurrence and prognosis. From 1998 to 2004, they performed surgery in 274 cases with ground-glass opacity features. They had a reference protocol by which a patient with a non-solid lesion 1.5 cm or larger in diameter would immediately undergo surgery; otherwise, patients would be monitored with TSHR CT every 3 months. Eighty-eight patients showed pure ground-glass opacity or non-solid lesions on TSHR CT images. Of these, 53 patients were female, and the median age was 63 years. Among the 54 lesions smaller than 1 cm, only one increased in size; another one disappeared and the others were stable. Among the 35 lesions 1 cm or larger in diameter, 43% increased in size or changed in density. Of the patients, 10% had a history of lung cancer, and most had tumors that increased in size, which indicates that those lesions might be metastases instead of double primary cancer. Examination of the surgical margin would not be meaningful in case of non-solid lesions; histopathological diagnosis of invasiveness seemed more important for these lesions. Lung cancers showing ground-glass opacity features on high-resolution CT images tend to have a less invasive nature. Limited surgical resection might be suitable for selected patients.

Ken Kodama, MD, (Osaka Medical Center for Cancer and Cardiovascular Diseases, Japan) presented the results of a prospective phase II study evaluating alternatives to resection as early intervention for small peripheral lung cancer from a single institute. Eligible patients exhibited peripheral lung cancer with c-T1N0M0 with primary lesions 2 cm or