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RESEARCH ARTICLE Open Access

Genetic and epigenetic characteristics of human multiple hepatocellular carcinoma

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Abstract

Background: Multiple carcinogenesis is one of the major characteristics of human hepatocellular carcinoma (HCC). The history of multiple tumors, that is, whether they derive from a common precancerous or cancerous ancestor or individually from hepatocytes, is a major clinical issue. Multiple HCC is clinically classified as either intratumor metastasis (IM) or multicentric carcinogenesis (MC). Molecular markers that differentiate IM and MC are of interest to clinical practitioners because the clinical diagnoses of IM and MC often lead to different therapies.

Methods: We analyzed 30 multiple tumors from 15 patients for somatic mutations of cancer-related genes, chromosomal aberrations, and promoter methylation of tumor suppressor genes using techniques such as high-resolution melting, array-comparative genomic hybridization (CGH), and quantitative methylation-specific PCR.

Results: Somatic mutations were found in *TP53* and *CTNNB1* but not in *CDKN2A* or *KRAS*. Tumors from the same patient did not share the same mutations. Array-CGH analysis revealed variations in the number of chromosomal aberrations, and the detection of common aberrations in tumors from the same patient was found to depend on the total number of chromosomal aberrations. A promoter methylation analysis of genes revealed dense methylation in HCC but not in the adjacent non-tumor tissue. The correlation coefficients (r) of methylation patterns between tumors from the same patient were more similar than those between tumors from different patients. In total, 47% of tumor samples from the same patients had an $r \geq 0.8$, whereas, in contrast, only 18% of tumor samples from different patients had an $r \geq 0.8$ ($p = 0.01$). All IM cases were highly similar; that is, $r \geq 0.8$ ($p = 0.025$).

Conclusions: The overall scarcity of common somatic mutations and chromosomal aberrations suggests that biological IM is likely to be rare. Tumors from the same patient had a methylation pattern that was more similar than those from different patients. As all clinical IM cases exhibited high similarity, the methylation pattern may be applicable to support the clinical diagnosis of IM and MC.

Background

Human hepatocellular carcinoma (HCC) is one of the leading causes of death in Asian countries. Unlike cancers that are prevalent in other developed countries, HCC is characterized by underlying viral etiologic factors, such as hepatitis B virus (HBV) and hepatitis C virus (HCV). In Japan, HCV infection is the most common cause of HCC. One characteristic of HCC is a high rate of tumor recurrence [1-4], owing to multiple carcinogenesis. Multiple carcinogenesis is uncommon, except in HCC and some types of lung cancer. Multiple HCC is classified as either intrahepatic metastasis (IM) or multicentric carcinogenesis (MC) based on clinicopathological criteria [5,6]. Some groups have reported that IM recurrence develops earlier than MC, which leads to a poorer prognosis for IM than for MC [2,7]. Therefore, surgery may not be warranted for recurrent metastatic nodules, whereas, for MC lesions, radical surgery should be initially attempted if a functional liver reserve is adequate.

Numerous studies have investigated the genetic aberrations in HCC [8]. Somatic mutations in genes, such as *TP53*, have frequently been observed. Recurring allelic gains and losses on 14 chromosome arms have been detected in more than 30% of HCC cases [9-11]. These observations have been confirmed using array-comparative

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genomic hybridization (CGH) [12-14]. In addition to these genetic changes, epigenetic changes have also been extensively analyzed. Dense methylation of cancer-related genes is a characteristic of HCC [15]. Geographic variations in methylation status indicate that environmental factors affect the methylation status of genes in HCC [15]. In addition, the aberrant hypermethylation has been observed in non-neoplastic liver cells from patients with hereditary hemochromatosis [16].

As previously described, a history of multiple tumors in a single patient has been an important clinical issue. If there were multiple genomic aberrations, the lineage of multiple tumors could be deduced from the patterns of the aberrations. Genetic and epigenetic factors have been examined for this purpose. These factors include p53 mutation status [17], HBV integration sites [18], chromosome aberration [19-22], and methylation status [23]; however, despite these reports, no consensus leading to clinical application has been established. This is not surprising because the biological and genetic bases of IM and MC remain obscure.

In this study, we analyzed 30 multiple tumors from 15 patients for somatic mutations of cancer-related genes or chromosomal aberrations (i.e., allelic gains and losses) and the promoter methylation status of cancer-related genes using the latest techniques, such as high-resolution melting [24], array-CGH [25], and quantitative methylation-specific PCR (QMSP) [26]. We examined whether multiple HCC has specific molecular changes that indicate the process of carcinogenesis. We also evaluated whether these changes could be applicable to the differentiation of IM and MC.

Methods

HCC samples

A total of 30 tumor tissues and adjacent non-tumor tissues were obtained from 15 HCC patients who underwent their first surgical operation between 1998 and 2006. Tissues were stored at -80°C until further use. DNA was extracted from the frozen tumor tissues and adjacent non-tumor tissues using a QIAamp DNA Micro kit (Qiagen, Valencia, CA). This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committee of Osaka Medical Center for Cancer and Cardiovascular Diseases. Informed consent was obtained from all of the investigated patients.

Mutation analysis

Mutation screening was performed using high-resolution melting on a LightScanner (Idaho Technology Inc., Salt Lake City, UT) [24] according to the manufacturer's protocol, which was then followed by direct sequencing of the PCR products. The primer sequences that were used for both assays are provided in Additional file 1, Table S1.

Analysis of chromosomal aberration

Array-CGH was performed using a 44K array (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol [25]. Gains and losses that spanned fewer than 100 probes were omitted from the results in order to make the output comparable to those from previous studies that were carried out using conventional CGH or microsatellite markers. The extraction of data from images was carried out using the Feature Extraction Software (Agilent Technologies), and gains and losses were identified using the DNA Analysis Software (Agilent Technologies): the ADM-2 algorithm was employed using 8.0 as the threshold and 0.3219 as the minimum absolute average log ratio for the region. The array-CGH data was submitted to NCBI GEO (accession number, GSE22635).

Methylation analysis

Genomic DNA was subjected to bisulfite treatment before methylation analysis, as previously described [27]. QMSP was performed as previously described [26] using TaqMan technology. The primer and probe sequences that were used are given in Additional file 1, Table S1. In order to prepare the positive control (i.e., 100% methylated DNA), we treated a mixture of genomic DNA from five lung cancer tissues with Sss I (CpG) methylase (New England Biolabs, Inc., Beverly, MA). In order to convert the nonmethylated cytosine residues into uracil, genomic DNA was treated with sodium bisulfite using the MethylEasy DNA Bisulphite Modification kit (TAKARA, Kyoto, Japan). Whereas a 5-methyl cytosine within the CpG islands remained unaltered, 4 μg of DNA was denatured by NaOH and modified by sodium bisulfite at 55°C for 12 hr. The DNA samples were then purified by isopropanol precipitation, washed with 70% ethanol, and resuspended in 50 μl water. The samples were then incubated at 72°C for 1 hr.

QMSP for 13 genes was performed using the 7500 Real-Time PCR System(Life Technologies, Carlsbad, CA). PCR was performed in a total volume of 20 μl, which consisted of 40 ng bisulfate-modified genomic DNA, 10 μl TaqMan Universal PCR Master Mix(Life Technologies, 0.25 μM of each primer, and 0.2 μM of the TaqMan probe. The primer and probe sequences that were used here are given in Additional file 1, Table S1. After an initial denaturation at 50°C for 2 min and 95°C for 5 min, 50 cycles at 95°C for 15 sec and 60°C for 1 min were performed.

The assays were repeated twice so as to confirm reproducibility, and the average was used for the subsequent data analysis. Data analysis was performed as

previously described [28]. The values of QMSP that were obtained from each sample were first normalized using the value of *β-actin* as an internal reference. The values of QMSP that were obtained from the positive control were also normalized using the value of *β-actin*. The percent of methylated reference (PMR) was calculated as 100 × (normalized value of the sample)/(normalized value of the positive control). For statistical analysis, the PMR values that were less than 0.01 were rounded up to 0.01. Subsequently, the PMR values were converted to logarithms for statistical analysis.

Results

Somatic mutation analysis

The samples from patients 1-9 included pairs of primary and recurrent tumors, whereas those from patients 10-15 included multiple primary tumors. Except for patient no. 11, all HCC patients had backgrounds of viral infection. All of the clinical information is presented in Table 1.

Genes subjected to the somatic mutation analysis - *TP53, CTNNB1 (β-catenin), CDKN2A*, and *KRAS* - were chosen using COSMIC (Catalog of Somatic Mutation in Cancer: http://www.sanger.ac.uk/genetics/CGP/cosmic/). The analysis was conducted using high-resolution melting and direct sequencing of the PCR products because direct sequencing often misses mutations on rare alleles; however, there was no discrepancy of results between two techniques. In our samples, no mutations were detected in *CDKN2A* or *KRAS*. As shown in Table 1, somatic mutations in *TP53* and *CTNNB1* were found to be associated with several tumors; however, tumors

Table 1 Summary of clinical information and experimental results.

patient	HCC_ID	Clin. Diag.	HBsAg	HCV-Ab	p53	beta-catenin	GMA	common aberration	r
1	2	MC	-	+			2.45	+	0.87
	4								
2	6	MC	+	-	R273H	S33C	0	-	0.63
	8								
3	10	MC	-	+			2.24	-	0.22
	12				Q129stop				
4	14	MC	-	+	R249S		8.49	+	0.7
	16				S183stop, E298stop				
5	18	MC	-	+		S33C	3.16	-	0.8
	21								
6	102	MC	-	+	Q245C		4.9	-	0.83
	104				R249S	Q34E			
7	106	IM	-	+			0	-	0.82
	108								
8	110	IM	-	+			8.94	+	0.82
	112								
9	114	MC	+	-			14.8	+	0.21
	116								
10	26*	MC	-	+			0	-	0.27
	27*								
11	29*	IM	-	-		Q34E	2	-	0.9
	30*					V22G, S33S, I35S			
12	32*	IM	+	-	R175H		0	-	0.87
	33*								
13	35*	MC	-	+	Y163N	D32G	10	+	0.63
	36*					T41I			
14	38*	MC	-	+	F278A		14.3	+	0.4
	39*								
15	41*	MC	+	-			1.41	-	0.76
	42*								

*, tumors dissected at the same operation. Others were sampled at different operations.
GMA, geometric mean of number of chromosomal aberrations; common aberrations, common chromosomal aberrations found in tumors from same patients; r, correlation coefficient of methylation patterns of tumors from same patients.

from the same patient did not share the same mutations. Furthermore, no mutations were found in the non-tumor counterparts (data not shown).

Chromosomal aberration analysis

Chromosomal aberrations (gains and losses) were analyzed using the 44K human genome array, and the results are given in Table 2. Chromosome gains and losses at a probe-level resolution are given in Additional file 2, Table S2. The overviews of the aberrations were similar to those that have been published in previous studies [10,12,14]: frequent gains with 1q (9/30 in Table 2), 8q (12/30), and 20q (5/30) and losses with 1p (5/30), 4q (9/30), 8p (9/30), 13q (6/30), 16q (7/30), and 17p (10/30). Chromosomal aberrations that are common to multiple tumors are an important signature for tracing their histories, in that they indicate that these multiple tumors share a common lineage. Because array-CGH is sufficiently refined to determine breakpoints, we excluded aberrations that had different breakpoints at both ends from common aberrations. Tumors from six patients had common aberrations (Table 2, common aberration). For example, patient no. 13 had common aberrations in 1p and 17q: tumors no. 35 and 36 were estimated to have derived from an ancestor that had these aberrations.

As shown in Table 2, the number of chromosomal aberrations varied as a function of tumor in different patients as well as in the same patient. Figure 1 depicts the geometric mean aberration number in each patient, grouped according to presence or absence of common aberrations. There is a marked difference in the numbers of aberrations between the two groups (Mann-Whitney test, $p < 0.01$). Thus, the identification of common aberrations depends on the total number of chromosomal aberrations.

Methylation analysis

The genes that were subjected to the analysis of promoter methylation were mostly tumor suppressor genes, which are methylation markers that are widely used in cancer studies. The gene set included all of the genes that have been used in recent related studies [16,23]. We performed QMSP using all of the tumor samples as well as adjacent non-tumor samples. The results (PMR) are given in Additional file 3, Table S3. In most cases, the amplification using non-tumor samples was less or far less than the detection level using HCC (Additional file 3, Table S3), confirming the cancer-specific methylation of the genes. In HCC, the degree of methylation differed as a function of the type of gene. Group A genes tended to have high PMR values, whereas group B genes tended to have diverse PMR values (Figure 2). QMSP has a wide dynamic range and is sufficiently sensitive to detect a methylation as low as 0.02%.

Because the methylation status that is described by the PMR value is not discrete, we compared the overall patterns by calculating the correlation coefficient (r). First, we calculated the r of log-converted PMRs using all of the possible combinations of tumors from different patients. The distribution of r is shown in Figure 3a. The distribution ranged from 0.96 to -0.14, and the median was 0.601. This is a control distribution that was obtained from sample pairs of completely different origins. The distribution of r of tumors from the same patient is shown in Figure 3b - this distribution is shifted to the right, suggesting a greater similarity in methylation patterns in tumors from the same patient. In total, 47% (7/15) of cases had an r that was greater than 0.8 (Figure 3b). In contrast, only 18% of tumors from different patients had an r that was greater than 0.8 (Figure 3a). The chance of more than seven cases for which r was more than 0.8 out of 15 sample pairs from different patients was deduced by randomly sampling 15 cases from the pool of pairs from different patients: 110 successes per 100,000 trials. The high similarity in methylation patterns in the same patient was, thus, statistically significant. It should be noted that all clinical IM cases had values of r that were greater than 0.8 ($p = 0.025$, Fischer's exact test) (Table 1). Despite the detailed examination of the methylation in individual genes, we could not find any rule for the high similarity. The high similarity of the overall methylation patterns is a potential indicator of IM.

Discussion

The origin of individual tumors in HCC is a major issue; that is, whether they are derived from a common precancerous or cancerous ancestor or individually from hepatocytes. We can deduce how individual tumors evolved from common ancestor cells by comparing the aberration patterns of multiple genetic aberrations. This could lead to the differential diagnosis of IM and MC, which is often important when making a therapeutic decision. The initial step of the analysis is to find molecular genetic features that are common to individual tumors. Many efforts have been made to determine common genetic changes [17-23] and to correlate them with a clinical diagnosis; however, as described earlier, there is still no consensus. In this report, we evaluated somatic mutations, chromosomal aberrations, and promoter methylation. The latest techniques, such as array-CGH and QMSP, were used for the first time for the analysis of multiple HCC in this study.

The occurrence of somatic mutations was too rare to identify common aberrations. Despite the initial effort to

Table 2 Results of array-CGH.

patient	HCC_ID	number of aberrations	geometric mean	common aberration	chrosome gain	chrosome loss
1	2	2	2.45	+	6p,8q	
	4	3			6p, 7q, 8q	
2	6	7	0	-		4q,8p,9p,9q,11q,16q,21q
	8	0				
3	10	5	2.24	-	4p,4q,17p,17q,22q	
	12	1			9q	
4	14	12	8.49	+	1q,11q	3pq,4q,8p,9pq,10q,11p,13pq,16q,17p,Yp
	16	6			14q,17q,19q	4q,11pq,13q
5	18	2	3.16	-	8q,Xq	
	21	5			7p	8p,12p,13q,17p
6	102	1	4.9	-	19p	
	104	24			1q,5p,5q,6p,8p,8q,11p,11q,20p,20q,Yp,Yq	1p,4p,4q,12p,12q,14q,15q,17p,18p,18q,21q,22q
7	106	0	0	-		
	108	18			5p,5q,6p,8q,13q,19p,19q,20p,20q,21q,22q,Xp,Xq,Yp	4q,8p,9p,13q
8	110	8	8.94	+	1q,8q,11pq,Yp,Yq	8p,16q,17p
	112	10			20q,Yp	1p,6q,8p,16p,16q,18q,Xp,Xq
9	114	11	14.8	+	2q,11q,22q	1p,1q,6q,8pq,10q,13q,16q,17p
	116	20			1q,6pq,7p,8q,13q,19q	1p,4q,6q,8p,9p,10p,12p,14q, 16p, 16q,17p,18q,19p,21q
10	26*	0	0	-		
	27*	0				
11	29*	1	2	-	8q	
	30*	4			1q,8q,Xq	10q
12	32*	1	0	-	20q	
	33*	0				
13	35*	25	10	+	1q,3p,3q,5p,5q,6p,6q,8p,8q,10p,10q,11p,11q 17q,18p,18q,19q,20p,20q,21q,Xp,Xq	4q,17p,Ypq
	36*	4			1q,17q	6q,17p
14	38*	12	14.3	+	1q,7pq,19q	1p,4q,5q,7q,8p,16q,17p,18p,18q
	39*	17			1q,3q,4p,8q,10q,19q,Xp,Xq	4q,7p,8p,9p,12p,13q,14q,17p,18q
15	41*	1	1.41	-	8q	
	42*	0				

*, tumors dissected at the same operation. Others were sampled at different operations. Common chromosomal aberrations are underlined.

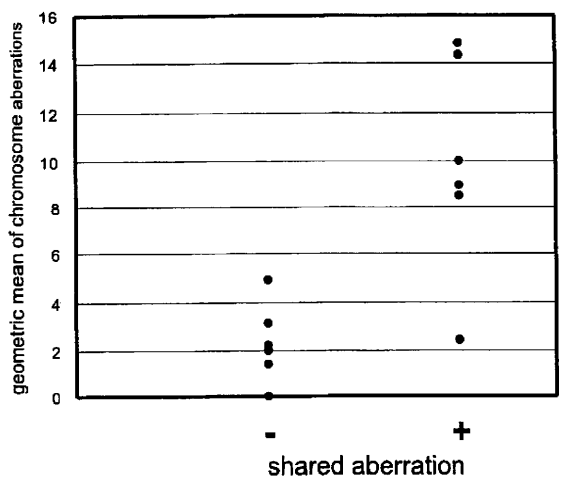

Figure 1 The relationship between the occurrence of chromosomal aberrations that are common to tumors from the same patient and the average number of aberrations. Dots represent cases. (+), Cases with common aberrations; (-), cases without common aberrations.

use p53 for the differential diagnosis [17], we found that it is mutated in only a few cases, which was similar to previous reports [29,30]. We found common chromosomal aberrations in six cases, wherein multiple tumors were likely to have derived from a common lineage; however, the occurrence of chromosomal aberrations differed within tumors, as well as among tumors from the same patient. In cases with infrequent chromosomal aberrations, it was difficult to deduce the history of tumors from the aberration pattern. Even tumors with common aberrations possessed different aberrations and were not clones. In contrast, we found, from quantitative analysis of CGH patterns, that there was no substantial heterogeneity in each tumor (data not shown). These observations suggest that biological IM tumors, that is, clonal tumors, were rare in comparison to biological MC tumors, that is, multiple tumors with different genotypes, which strongly contrasts with recent observations in other cancers that have been obtained by large-scale sequencing. For example, recent work in colorectal cancer has demonstrated that more than 90% of somatic mutations were simultaneously present in different malignant tumors; that is, a primary tumor versus its metastases or a primary tumor versus a recurrent tumor in the same patient [31].

As previously described, the choice of therapy often depends on the clinical diagnosis of IM and MC [5,6]. Multiple HCC is diagnosed as IM when the primary tumor is moderately or poorly differentiated, and multiple tumors appear within two years after surgery. The diagnosis of MC is achieved when multiple tumors are highly differentiated and appear with hepatitis or cirrhosis; however, these criteria have no direct correlation with the process of carcinogenesis. Therefore, an exploration of the molecular genetic differences between IM and MC does not have a solid scientific basis. Thus, it is not surprising that there has been no consensus in

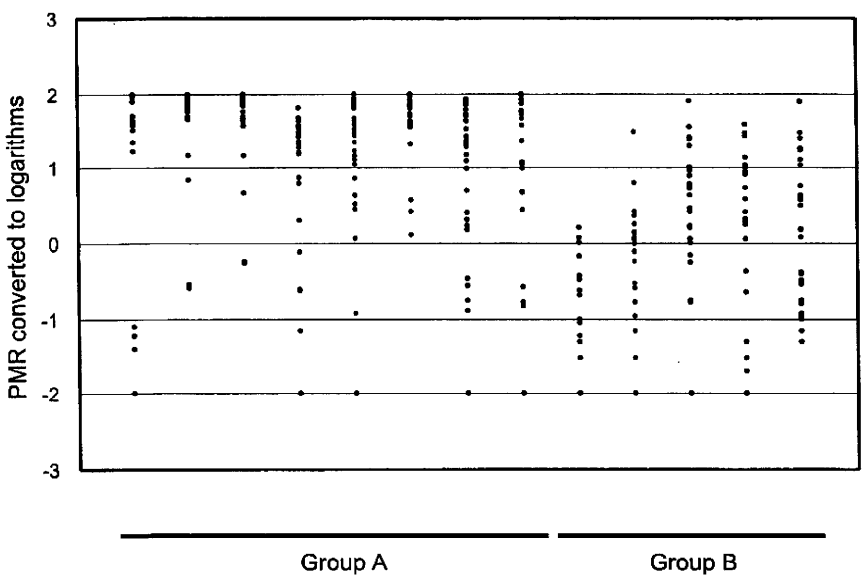

Figure 2 The promoter methylation of genes. Vertical axis, PMR values that are converted to common logarithms; horizontal axis, genes that are subjected to promoter methylation analysis.

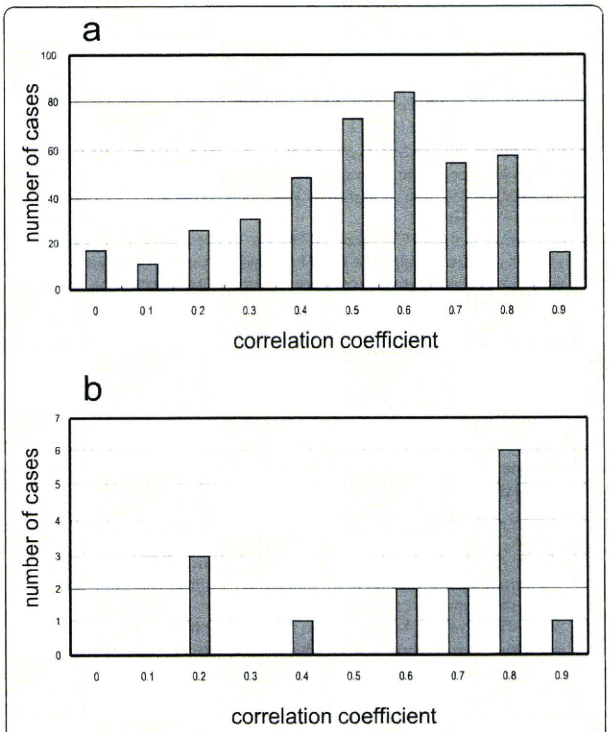

Figure 3 A histogram of correlation coefficients of methylation patterns. Vertical axis, number of cases; horizontal axis, correlation coefficient ranges. For example, 0.8 represents values that range from 0.8 to 0.9. "0" includes cases from -0.14 to 0. a) All possible combinations of tumors from different patients; b) tumors from the same patient.

the molecular diagnostic criteria for IM and MC. Our data concerning somatic mutations and chromosomal aberrations suggest that biological IM is likely to be rare. Difficulty in the molecular differentiation of IM and MC is at least partly due to the rarity of biological IM.

All IM cases exhibited a similar methylation pattern. Unlike genetic changes, epigenetic changes were not necessarily irreversible. Here, a similar methylation pattern for multiple HCC would reflect the environmental factors that surrounded their development rather than their derivation from a common ancestor because the data concerning somatic mutations and chromosomal aberrations suggest the rarity of biological IM. Although confirmation with a larger number of patients is still required, the methylation pattern may be useful in the clinical diagnosis of marginal cases.

In general, current techniques do not offer adequate information on the carcinogenesis of multiple HCC. There is also a possibility that the negative results are due to the small sample size. Recently, sequencers based on a new principle have appeared, and the rate of sequence data production has improved by more than 100 times and is still increasing [32]. The lineage of multiple tumors and liver tissues and the process of carcinogenesis will be identified when the somatic mutations are revealed by the entire genomic sequencing of multiple HCC.

Conclusions

The overall scarcity of common somatic mutations and chromosomal aberrations suggest that biological IM is likely to be rare. Tumors from the same patient had a methylation pattern that was more similar than tumors from different patients. Because all clinical IM cases were highly similar, methylation patterning may be applicable to support the clinical diagnosis of IM and MC.

Additional material

Additional file 1: Table S1.
Additional file 2: Table S2.
Additional file 3: Table S3.

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Authors' contributions
KT designed and carried out all of the molecular genetic studies. TY and YS collected the tumor and normal tissues and are responsible for the clinical components of the study. KK designed the study, participated in its design and coordination, and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Sublobar Resection Provides an Equivalent Survival After Lobectomy in Elderly Patients With Early Lung Cancer

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Background. Sublobar resection is indicated for early-stage non-small cell lung cancer in patients with a perioperative risk associated with impaired medical conditions. This study was conducted to investigate the clinical impact of this procedure in the elderly.

Methods. The patients who underwent complete resection for stage IA non-small cell lung cancer from 1990 and 2007 were enrolled (n = 764). Two age groups were defined as elderly (≥75 years) and younger (<75 years) patients. The 5-year survival, recurrence, and postoperative complications after sublobar resection were compared with those after standard lobectomy according to age group.

Results. There were 133 elderly patients (79 standard lobectomies and 54 sublobar resections) and 631 younger patients (539 standard lobectomies and 92 sublobar resections). While the 5-year survival after sublobar resection was significantly inferior to that after standard lobectomy in the younger group (64.0% and 90.9%, respectively, $p < 0.0001$), however, no substantial difference was observed in the elderly (67.6% and 74.3%, $p = 0.92$). Locoregional recurrence rates were higher in patients after sublobar resection than those after standard lobectomy in both the elderly (11.1% vs 1.3%) and the younger (12.0% vs 1.5%) groups. No significant difference in postoperative complications was observed between the types of surgery in the elderly.

Conclusions. Sublobar resection for stage IA is considered to be an appropriate treatment in the elderly patients as this procedure provides an equivalent long-term outcome in comparison with lobectomy. A larger scale study with matching patients is necessary to confirm the noninferiority of sublobar resection in comparison with standard lobectomy in this population.

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Lung cancer is the leading cause of cancer-related deaths in many countries and patients older than 80 years account for 14% of all lung cancers [1, 2]. The number of elderly lung cancer patients is increasing rapidly worldwide. Comorbid illness and adverse medical conditions due to aging is a significant concern to treat elderly patients with lung cancer [3]. Lobectomy is the current standard treatment for early-stage non-small cell lung cancer (NSCLC) in the general population. Sublobar resection such as wedge resection and segmentectomy could be indicated in patients with stage I NSCLC, who may tolerate operative intervention but not a lobar or greater lung resection because of comorbid disease or decreased cardiopulmonary function [4]. When treating elderly patients, decisions regarding the treatment strategy, lobectomy, or sublobar resection, must therefore carefully balance the risks of postsurgical morbidity and mortality with those affecting cancer recurrence and long-term survival.

This study was conducted to investigate the clinical impact of sublobar resection in the elderly patients in comparison with their younger counterparts. The short-term and long-term outcomes after sublobar resection for stage IA NSCLC were compared with those after standard lobectomy according to the age group.

Patients and Methods

Patients

This study conducted a retrospective review of 984 patients who underwent complete resection for stage IA NSCLC at the Osaka Medical Center for Cancer and Cardiovascular Diseases from January 1991 to December 2007. The ethics committee gave its approval for the publication of this retrospective study with a waiver of informed consent (N0.1003175124) from the individual patients. The institutional prospective database of the general thoracic department included clinicopathologic variables and the postoperative clinical course. The pri-

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mary variables were gender, age, smoking status, preoperative treatment, comorbidity, surgical procedure, curability, diameter of surgical tumor specimen, histology, and pathologic (p) stage. The outcomes included postoperative complications, type of recurrence, and survival time. The type of surgery was categorized into two groups according to the extent of the pulmonary resection; standard surgery including a lobar or greater lung resection and sublobar resection such as segmentectomy and wedge resection. Any patients undergoing sublobar resections with a radical intent for the treatment of small-sized (2 cm or smaller) noninvasive carcinoma (n = 220) were excluded from the study. Definition of radical intent sublobar resection was described in detail in the previous report [5]. Briefly, the indications for radical intent sublobar resection were determined according to the diameter of the nodule and the percentage of ground-glass opacity on high-resolution computed tomographic (CT) scans. Finally, 618 patients who had standard surgery and 146 patients who underwent sublobar resection were enrolled in the study. Two age groups were defined as elderly (≥75 years) and younger (<75 years) patients.

Preoperative and Intraoperative Evaluation and Staging

The preoperative evaluation included a detailed clinical history and physical examination, chest radiography, chest and upper abdominal CT scans, brain magnetic resonance imaging, and bone scintigraphy or fluorodeoxyglucose-positron emission tomography scan for staging and assessment of respectability. All patients were staged intraoperatively and pathologically according to the sixth TNM (tumor-nodes-metastasis) classification at the time of surgery, and the TNM descriptions were converted to the seventh edition which has been recently updated [6]. Hilar and (or) mediastinal lymph nodes were sampled or systematically dissected during lobectomy or segmentectomy to evaluate for the possibility of occult nodal metastases. On the other hand, only swollen nodes were sampled in the patients who underwent wedge resection. A lavage cytologic examination was routinely used to assess the resection margins for tumor presence intraoperatively, as previously reported [7].

Reasons for Selecting Sublobar Resection and Postoperative Complications

Comorbid diseases and postoperative complications were diagnosed by laboratory, radiologic, and physiologic examinations. The reasons for selecting sublobar resection were defined as the following: insufficient pulmonary function or chronic lung diseases (abnormal spirometry test and [or] apparent interstitial shadow or emphysema detected by chest CT); insufficient cardiac function or cardiovascular diseases; previous lung surgery (greater than lobectomy) or active multiple lung cancer; cancer history; and diabetes mellitus. Multiple reasons were allowed. Complications were defined as the following: life-threatening complications which required any kind of emergent interventional treatment, or transfer to an intensive care unit; major complications were those that were potentially life threatening but did not require emergency intervention; and minor complications included those that required therapy and a prolonged hospital stay.

Recurrence of the Disease and Survival

Recurrence was diagnosed by daily clinical practice and defined as locoregional if it occurred within the same lobe, the mediastinal lymph nodes, or the hilum. All other types of recurrence were categorized as distant recurrence. The survival time was measured from the date of surgery to the date of the most recent follow-up examination or the date of death. The patients lost to the follow-up within ten years after surgery were censored at the date of last contact with the institution.

Statistical Analysis

The χ^2 test or Fisher exact test was used to compare the frequencies of categoric measures. Survival was calculated by the Kaplan-Meier method and differences in survival were assessed by a log-rank analysis. To adjust the effect of death due to other causes and to control the difference in the age and gender distribution between the sublobar resection group and the standard surgery, we calculated the relative survival and performed an age stratified analysis. The relative survival was estimated using the maximum-likelihood approach for individual data with the publicly available STATA program *strel* (StataCorp, College Station, TX) [8, 9]. The relative survival was the ratio of the observed survival rate in the patient group and the expected survival rate derived from the population life tables after matching for the age, calendar year, and sex. It can be interpreted as the survival from cancer after adjustment for other causes of deaths. A multivariate analysis for prognostic factors was performed using the Cox proportional hazard regression model. The p values less than 0.05 were considered to be statistically significant.

Results

Patient Characteristics

Table 1 summarizes the patient characteristics from the age groups. The tumor histology was as follows: adenocarcinoma in 637 patients; squamous cell carcinoma in 105; large cell carcinoma in 11; adenosquamous carcinoma in 8; and 3 pleomorphic or sarcomatoid carcinoma. The standard surgery group in the total cohort included 2 pneumonectomies, 12 bilobectomies, and 604 lobectomies, while the sublobar resection group included 90 segmentectomies and 56 wedge resections. There were more males ($p = 0.0189$), more squamous cell carcinomas ($p = 0.001$), and more ex-smokers or current smokers ($p < 0.0001$) in comparison with those in the standard lobectomy group. The histology and smoking status were significantly different between the types of surgery in the elderly patients (≥75 years of age). All of the patients had macroscopically negative surgical margin. Operative mortalities, which included deaths within the first 30

Table 1. Patients' Characteristics From the Overall Cohort and Each Age Group

	Younger (<75 Years)			Elderly (≥75 Years)		
Characteristic	Standard (n = 539)	Sublobar (n = 92)	*p* Value	Standard (n = 79)	Sublobar (n = 54)	*p* Value
Age (years)						
Median (mean)	64	68		77	78	
Range	35–74	38–74	<0.0001	75–87	75–84	0.2080
Gender						
Male	258	72	0.0189	45	39	0.1074
Female	281	20		34	15	
T stage						
T1a (≤20 mm)	198	46	0.1885	25	22	0.4976
T1b (>20 mm)	341	46		54	32	
Histology						
Adenocarcinoma	468	67	0.0010	66	36	0.0400
Squamous cell carcinoma	59	19		10	17	
Others	12	6		3	1	
Surgery						
Pneumonectomy	2	0	NA	0	0	NA
Lobectomy	537	0		82	0	
Segmentectomy	0	57		0	33	
Wedge	0	35		0	21	
Smoking status						
Ex- or current smoker	256	65	<0.0001	44	40	0.0481

NA = not applicable.

days after surgery or during the same hospitalization, were not recorded in this study.

Reasons for Selecting Sublobar Resection

The reasons for selecting sublobar resection are listed in Table 2. Insufficient pulmonary function or chronic lung disease was the most common and insufficient cardiac function or cardiovascular disease was the second. All of the patients with cancer history were examined thoroughly before pulmonary resection to confirm that they had no active recurrence or metastatic lesion other than primary lung cancer.

Survival Analyses

The 5-year survival rates were 84.6% for the overall cohort, 89.3% for the standard surgery, and 65.2% for the sublobar resection. The long-term survival after sublobar resection was significantly inferior to that after the standard surgery ($p = 0.0015$, Fig 1A). A multivariate analysis showed advanced age, sublobar resection, and nonadenocarcinoma to be independent significant unfavorable factors for the overall survival (Table 3). We further calculated the relative survival and performed an age-stratified analysis in each age group between the types of surgery. The 5-year relative survival rates of the younger patients were 90.9% (95% confidence interval [CI], 87.7% to 93.3%) for the standard surgery group and 64.0% (95% CI, 51.9% to 73.8%) for the sublobar resection group (Fig 1B). On the other hand, the difference between the types of surgery disappeared in the elderly patients (Fig 1C). The 5-year relative survival rates of the elderly patients were 74.3% (95% CI, 60.8% to 83.7%) for the standard surgery and 67.6% (95% CI, 51.7% to 79.3%) for the sublobar resection. To examine the survival effect between the types of surgery according to age group, we divided the patients into the following four groups: (I) younger patients who underwent standard lobectomy; (II) younger patients who underwent sublobar resection; (III) elderly patients who underwent lobectomy; and (IV) elderly patients who underwent sublobar resection. Thereafter, we calculated the hazard ratios for death of each patient group based on a multivariate Cox proportional model. Group I was used as a control group. As shown in Figure 2, while the hazard ratio of group II was 2.83 (95% CI, 1.84 to 4.35) as compared with the control group, the hazard ratio of group IV (2.64; 95% CI, 1.61 to 4.31) was similar to that of group III (2.97; 95%CI, 1.79 to 4.95).

Table 2. Reasons for Selecting Sublobar Resections

Reasons	Younger (<75 years) (n = 92) (%)	Elderly (≥75 years) (n = 54) (%)
Insufficient pulmonary function or chronic lung diseases	50 (54.3)	25 (46.3)
Insufficient cardiac function or cardiovascular diseases	22 (23.9)	20 (37.0)
Previous lung surgery or multiple lung cancer	14 (15.2)	7 (13.0)
Cancer history	9 (9.8)	3 (5.5)
Diabetes mellitus	4 (4.3)	6 (11.1)
Other	4 (4.3)	3 (5.5)

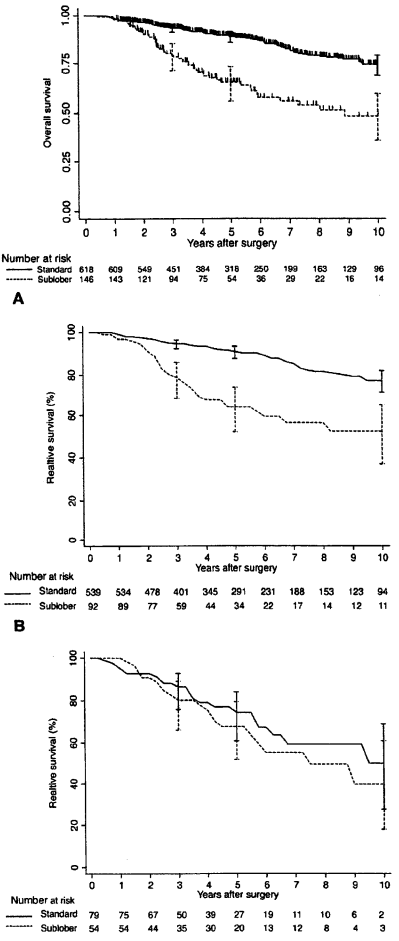

Fig 1. Postoperative survival curves according to the types of surgery (standard surgery or sublobar resection) with 95% confidence intervals at 3, 5, and 10 years after surgery. (A) The overall survival of the overall cohort (all ages); (B) the relative survival of the younger patients (<75 years); and (C) the relative survival of the elderly patients (≥75 years).

Table 3. Multivariate Analysis of Survival: Cox Proportional Hazard Model

Variable	HR	95% CI	*p* Value
Age	1.045	1.023–1.068	<0.0001
Operative procedure			
Standard surgery	ref		
Sublobar resection	1.835	1.261–2.670	0.0015
Histology			
Adenocarcinoma	ref		
Nonadenocarcinoma	1.739	1.160–2.604	0.0074
Gender			
Female	ref		
Male	1.324	0.786–2.231	0.2919
T stage			
T1a	ref		
T1b	1.018	0.716–1.447	0.9196
Smoking status			
Nonsmoker	ref		
Ex- or current smoker	1.178	0.687–2.020	0.8490

CI = confidence interval; HR = hazard ratio.

Postoperative Complications in the Elderly Patients

Thirty-five of the elderly patients (26.3%) experienced postoperative complications (Table 4). Life-threatening complications included two cases of acute myocardial infarction and one drug-induced anaphylactic shock. The occurrence of a life-threatening or a major complication was not associated with the types of surgery ($p = 0.3146$).

Recurrence of the Disease

Any recurrences of the disease during the follow-up period are summarized in Table 5. The percentages of distant metastasis ranged from 11.3% to 13.0% regardless the types of surgery or the patients' age. On the other hand, the local recurrence in the overall cohort apparently occurred more commonly in the patients who had

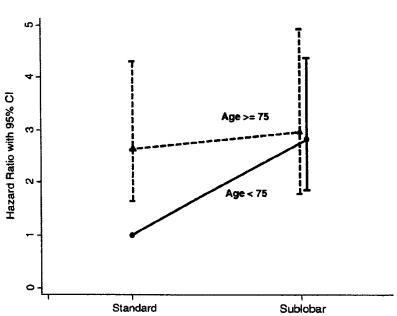

Fig 2. Comparison of the hazard ratio between standard surgery and sublobar resection in younger patients (solid line) and elderly patients (dashed line). (CI = confidence interval.)

Table 4. Postoperative Complications in the Elderly Patients

Complications	Standard (n = 79)	Sublobar (n = 54)
Life threatening (%)	1 (1.3)	2 (3.7)
Major (%)	4 (5.1)	5 (9.3)
Minor (%)	15 (19.0)	8 (14.8)

sublobar resection than in patients who had standard surgery (11.6% and 1.5%, respectively).

Comment

Removing the entire lobe, which contains the primary tumor, provides the highest probability for a complete resection of the disease including tumor cells spreading into adjacent pulmonary parenchyma and occult metastasis in the regional lymph nodes. However, surgeons often hesitate to recommend lobectomy for patients under comorbid conditions or with poor pulmonary function. Instead, sublobar resection such as wedge resection and segmentectomy are often offered to those patients in daily clinical practice to reduce surgical stress and to preserve more pulmonary function. This study revealed that sublobar resection provided an equivalent long-term outcome to that of lobectomy in the elderly patients.

The difference in the survival after lobectomy and sublobar resection has been debated even after one randomized trial demonstrated that sublobar resection for stage IA had a higher local recurrence rate and a shorter survival [10]. Nationwide retrospective studies in Japan and the US identified extent of surgical resection as a significant prognostic factor after curative resection for stage IA [11, 12]. These findings support the fact that lobectomy is the gold standard for stage IA lung cancer. On the other hand, studies focusing on elderly patients with stage IA revealed that anatomic segmentectomy was associated with reduced surgical risks and comparable oncologic efficacy [13]. Furthermore, according to the data from The National Cancer Registry in the United States, the statistical difference between survival curves of lobectomies and limited resections for stage I or II disappeared at 71 years of age [14]. In addition, the Japanese Joint Committee of Lung Cancer Registry also found no significant difference in the survival after lobectomy or sublobar resection for c-stage I of octogenarian patients [15].

Previously, several reports have demonstrated that sublobar resection was not inferior to standard lobectomy regarding the prognosis of patients with small-sized NSCLC [16, 17]. When comparing the outcomes of sublobar resection with that of lobectomy, it is important to mention the peripheral nodules, which are identified as a shadow containing ground-glass opacity by CT scanning. Most of such nodules are histologically diagnosed as early adenocarcinoma or minimally or noninvasive bronchioloalveolar carcinoma. The long-term result of this disease is excellent and the 5-year survival rate reaches to more than 96% even after sublobar resection [18, 19]; in contrast, the 5-year survival of NSCLC at stage IA is reported to be 83.9% [20]. Therefore, in order to elucidate the outcomes after sublobar resection in compromised patients, it is necessary to exclude the patients who underwent wedge resection or segmentectomy for this distinct subset of early-stage lung cancer. Otherwise, the outcome of the sublobar resection group might be spuriously superior to that of patients who underwent the same treatment due to their impaired medical condition. We have established institutional criteria based on the CT findings to indicate a sublobar resection with a radical intent for peripheral noninvasive carcinoma [5]. These criteria defined the patients who underwent sublobar resection due to the patients' medical and (or) physiologic condition. Therefore, the results of sublobar resection shown in this study were solely derived from patients who demonstrated medically impaired conditions.

The long-term results of p-stage IA based on the new staging system in the present study, 89.0% after the standard surgery and 65.3% after sublobar resection without any operative mortality, were satisfactory. As previously reported [12], age proved to be an independent predictor of survival in patients with stage IA. The patients were stratified by age group to eliminate an effect of the different distribution of the patients' age between the standard surgery and the sublobar resection. Furthermore, to consider the effect of background mortality, the relative survival was calculated and the prognosis was compared adequately between the types of surgery. One of the important findings in this study is that sublobar resection was a strong independent predictor for shortened survival in the overall cohort, but the types of surgery, standard or sublobar resection, did not affect the survival in the elderly patients. Multivariate analysis revealed that the unfavorable effect of sublobar resection on survival was apparent in the younger patients whereas the hazard ratio of sublobar resection was similar to that of standard lobectomy among elderly patients.

Following the equivalent survival in the elderly, postoperative complications were also studied. The occurrence of complications after sublobar resection did not increase in comparison with that after standard lobectomy even though the patients in the sublobar group were compromised. The reduced surgical intervention using lesser extent of pulmonary resection may contribute to this favorable result. The types of recurrence were

Table 5. Recurrence of the Disease From the Overall Cohort and Each Age Group

	Younger (<75 years)		Elderly (≤75 years)	
Type of recurrence	Standard (n = 539)	Sublobar (n = 92)	Standard (n = 79)	Sublobar (n = 54)
Locoregional (%)	8 (1.5)	11 (12.0)	1 (1.3)	6 (11.1)
Distant (%)	61 (11.3)	12 (13.0)	10 (12.7)	7 (13.0)

associated with the types of surgery but not with the patients' age group. It should be noted that the rate of locoregional recurrence was much higher in the patients after sublobar resection than in those after standard surgery and that the rate of distant metastasis did not increase in the patients who underwent sublobar resection.

The strengths of this study are that a single institutional study provided complete clinical and pathologic information, homogeneous treatment strategy, and well-controlled surgical quality. There are limitations that need to be acknowledged. The period of patient accrual was relatively long. The patients who underwent surgery from 1991 to 2007 were enrolled into the study, although the treatment strategy for stage IA did not change during this period. In addition, the number of elderly patients was smaller than that of the younger population. Secondly, sublobar resection cannot provide as much information for a final staging as lobectomy. Third, we should recognize that such retrospective analyses were inherently affected by predilections for several patients' factors between the types of surgery and the selection bias associated with surgical procedure chosen by thoracic surgeons in this study. There were more male patients, more nonadenocarcinoma, and more smokers in the sublobar group. These are known to be unfavorable prognostic factors in the patients with stage IA NSCLC. It should be noted that survival after sublobar resection was equivalent to that after lobectomy in the elderly.

In conclusion, sublobar resection in the elderly provides a long-term outcome which is equivalent to that obtained after standard lobectomy. This finding suggests that this procedure is considered to be an appropriate treatment strategy for elderly patients with stage IA NSCLC. A larger scale study with patients matched for the principal factors is necessary, however, to confirm the noninferiority of sublobar resection to standard lobectomy in this population.

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INVITED COMMENTARY

Okami and colleagues [1] have presented a retrospective review on the survival of elderly patients (age > 75) with stage 1A nonsmall cell lung cancer treated with a sublobar resection compared with elderly patients treated with a standard lobectomy between 1999 and 2007. There were 133 elderly patients in the study with 79 patients treated

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Follow-up papers - Thoracic oncologic

Clinical value of F18-fluorodeoxyglucose positron emission tomography-computed tomography in patients with non-small cell lung cancer after potentially curative surgery: experience with 241 patients

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Abstract

Objectives: F18-fluorodeoxyglucose positron emission tomography-computed tomography (FDG-PET/CT), which allows differentiation between malignant and benign lesions based on difference in tissue glucose metabolism, has become increasingly important in lung cancer diagnosis. This study examined the clinical value of FDG-PET/CT in a large number of patients with non-small cell lung cancer (NSCLC) after potentially curative surgery. **Methods:** Four hundred and ninety FDG-PET/CT of 241 patients (143 males and 98 females; age range 38–87 years; mean 68.0 years) between May 2006 and February 2008 were retrospectively evaluated. All the 241 patients had undergone potentially curative surgery for NSCLC >6 months before FDG-PET/CT and their pathologic stages were stage I and II according to the tumor-node-metastasis (TNM) classification. A final diagnosis of recurrence was confirmed by histologic or cytologic examination of the disease or by clinical and radiologic follow-up image analysis. Confirmation of recurrence-free status was based on a clinical and radiologic image analysis follow-up period of at least 12 months with no evidence of active malignancy. The diagnostic performance of FDG-PET/CT was evaluated. Details of false results and incidental detection of diseases other than recurrent lung cancer by FDG-PET/CT was also analyzed. **Results:** Recurrences were confirmed in 35 (15%) patients, and 206 patients (85%) had no evidence of recurrence. FDG-PET/CT correctly diagnosed recurrence in 34 of 35 patients and provided true negative findings in 198 of 206 patients who had no evidence of recurrence (sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of 97%, 96%, 96%, 81%, and 99%, respectively), indicating a high diagnostic performance. However, one patient had false negative studies and eight patients had false positive studies; misdiagnosis was more frequently in intrathoracic sites associated with postoperative changes. Malignancies other than recurrence were detected in nine of all 241 patients (4%) including five second primary lung cancers. **Conclusions:** The present study demonstrated the high diagnostic performance of FDG-PET/CT in detecting recurrences in a large group of patients with NSCLC after potentially curative surgery. FDG-PET/CT is useful not only for diagnosis of recurrence but also for detection of other diseases.

Keywords: Positron emission tomography; Lung neoplasms; Follow-up studies

1. Introduction

Lung cancer is the leading cause of cancer-related deaths in the Western world [1], and non-small cell lung cancer (NSCLC) accounts for ~85% of the cases. Surgery remains the best treatment for NSCLC, if curative resection is expected. Unfortunately, many patients who undergo potentially curative surgery eventually develop recurrences. Although no conclusive data support the survival benefits of earlier detection of recurrence or start of treatment for recurrent disease, early and accurate diagnosis of recurrence is important for selection of optimal therapy.

F18-fluorodeoxyglucose positron emission tomography (FDG-PET) has become increasingly important in the diagnosis of lung cancer. FDG-PET allows differentiation between malignant and benign lesions based on differences in glucose metabolism between normal and cancer tissues [2, 3]. Previous studies have demonstrated that FDG-PET is more accurate than computed tomography (CT) for the diagnosis and staging of NSCLC [4, 5]. The main disadvantage of FDG-PET is the poor quality of the anatomic information. To overcome this disadvantage of FDG-PET, new imaging systems using integrated FDG-PET/CT were developed recently.

Several groups reported the high diagnostic performance of the FDG-PET in suspected recurrence after definitive treatment [6–9]. Recently, Takenaka and co-workers [10]

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directly compared diagnostic capabilities of FDG-PET/CT and standard radiologic examination for assessment of recurrence in 92 postoperative NSCLC patients. In their series, 12 patients with recurrent lung cancer were included and they concluded that FDG-PET/CT can be used for assessment of postoperative recurrence in NSCLC patients with accuracy as good as that of standard radiological examinations. However, clinical study which includes large number of patients has not been conducted so far. In the present study, the diagnostic performance of FDG-PET/CT in large scale patients with NSCLC after potentially curative surgery was evaluated retrospectively. Details of false results and incidental detection of diseases other than recurrent lung cancer by PET/CT was also analyzed.

2. Patients and methods

2.1. Patient eligibility

In our hospital, FDG-PET/CT was performed in NSCLC patients after potentially curative surgery generally when the patient meets the following criteria. (1) New clinical symptoms or signs are observed. (2) Recurrence is suspected based on new abnormal or equivocal findings by conventional imaging modalities. (3) Elevation of serum tumor markers is observed. Serum tumor markers are routinely checked every 3–6 months after surgery. (4) Even if there are no unfavorable postoperative events, FDG-PET/CT is performed as a routine medical checkup approximately 12, 24 and 36 months after surgery or when the patient desires to undergo FDG-PET/CT.

Consecutive patients who underwent FDG-PET/CT between May 2006 and February 2008 in our hospital were evaluated retrospectively. Patients were eligible for this study if they had undergone potentially curative surgery for NSCLC >6 months before a follow-up. FDG-PET/CT and their pathologic stages were stage I and II according to the tumor-node-metastasis (TNM) classification. Patients were excluded from analysis if recurrence had been already confirmed by either biopsy or conventional imaging modalities before FDG-PET/CT. Patients who had another primary cancer at the time of surgery or diabetes mellitus were also excluded.

A total of 243 patients with 493 FDG-PET/CT studies were entered, however, two patients were lost to follow-up and excluded from the analysis. The final analysis included 241 patients (Table 1) with 490 FDG-PET/CT studies. Two FDG-PET/CTs were performed in 155 patients and three FDG-PET/CTs in 47 patients. The time interval between initial surgery and first FDG-PET/CT ranged from 6 to 215 months (median 25 months). The major histopathological cancer type was adenocarcinoma followed by squamous cell carcinoma (SCC) and other miscellaneous types (Table 1). The pathological stage after surgery according to the 1997 update of TNM classification was stage IA in 122 patients, IB in 61, IIA in 17, IIB in 41. The majority of patients underwent surgery alone as the primary treatment, though some underwent radio- or chemoradiotherapy followed by surgery, or surgery followed by adjuvant chemotherapy (Table 1).

Table 1
Clinical characteristics of the patient population

Characteristics	Number of patients
Sex	
Male	143
Female	98
Age (years)	
Mean	68.0
Range	38–87
Histopathology	
Adenocarcinoma	176
Squamous cell carcinoma	48
Other histopathological types*	17
Pathologic stage after surgery**	
IA	122
IB	61
IIA	17
IIB	41
Initial treatment	
Surgery alone	183
Induction radio- or chemoradiotherapy followed by surgery	5
Surgery followed by adjuvant chemotherapy	53
Interval between surgery and PET/CT (months)	
Median	25
Range	6–215

*Other histopathological types of NSCLC include adenosquamous carcinoma, large cell carcinoma.

**Stage of disease was defined according to the 1997 update of TNM criteria established by UICC.

PET/CT, positron emission tomography-computed tomography; NSCLC, non-small cell lung cancer; TNM, tumor-node-metastasis.

PET/CT was performed for some unfavorable postoperative events in 78 patients (suspected recurrence in nine patients based on onset of new clinical symptoms/signs, suspected recurrence in 31 patients based on new abnormal or equivocal findings by conventional imaging modalities, and suspected tumor recurrence in 38 patients due to high serum tumor markers). Table 2 provides more details of the reasons for performing FDG-PET/CT for suspected recurrence of the disease. In contrast, FDG-PET/CT was performed as a routine postoperative medical checkup in the remaining 163 patients. In 132 patients (81%) of this group, FDG-PET/CT was performed 6–36 months after surgery.

2.2. FDG-PET/CT imaging

Patients were asked to fast, except for glucose-free oral hydration, for at least 5 h before the injection of ^{18}F-FDG (3.5 MBq/kg body weight). After injection of the tracer, patients were kept lying comfortably on the bed. No urinary bladder catheterization was performed and no oral muscle relaxants were administered. Whole-body PET/CT fusion scanning was performed 1 h after the injection, using a PET/CT system (Discovery LS, General Electric Medical Systems; Biograph Duo LSD, Siemens-Asahi Medical Technologies). PET, CT, and fused PET/CT images were available for review, displayed in axial, coronal, and sagittal planes. The FDG uptake of tumor was visually compared with that of the surrounding tissue in areas devoid of prominent artifacts and overlapping increased FDG uptake organs.

Table 2
Reasons for conducting PET/CT for suspected recurrence of disease

Reason	Number of patients
Clinical signs and symptoms	9
Loss of body weight	3
Chest or back pain	4
Bloody sputum	1
Palpation of tumor	1
Abnormal or equivocal findings by conventional imaging	31
Lung nodules	14
Mediastinal lymphadenopathy	7
Pleural effusion	2
Inconclusive findings in surgical field	3
Other findings	5
Elevated tumor marker	38
CEA	32
CYFRA	2
CA19-9	2
SCC	1
NSE	1

CA19-9, carbonhydrate antigen 19-9; CEA, carcinoembryonic antigen; CYFRA, cytokeratin 19 fragment; NSE, neuron-specific enolase; SCC, squamous cell carcinoma antigen; PET/CT, positron emission tomography-computed tomography.

2.3. Interpretation of FDG-PET/CT

A team of experienced radiologists interpreted the FDG-PET/CT with knowledge of the patient's clinical history and previous imaging findings. Every site of increased FDG uptake was classified as malignant, equivocal, or benign based on the shape, size, and intensity of the uptake. A site of increased FDG uptake was defined as benign if it was located in an area of known physiologic distribution of the FDG or in a known non-malignant process, such as inflammation. A site of increased FDG uptake was defined as malignant if it was seen in areas unrelated to physiological or benign processes with higher intensity of the uptake than that of the surrounding tissues. Other sites of increased FDG uptake that could not be clearly characterized were defined as equivocal. An FDG-PET/CT study with at least one site defined as malignant was interpreted as positive. An FDG-PET/CT study with all sites defined as equivocal or benign was interpreted as equivocal or negative, respectively. An FDG-PET/CT study was interpreted as negative when no site of abnormal FDG uptake was observed. A site of increased FDG uptake which is considered to be other diseases than recurrent lung cancer, is also evaluated.

2.4. Analysis of FDG-PET/CT studies

Patient-based analysis was employed in this study. A final diagnosis of recurrence was confirmed by histopathological or cytologic examination of the disease or by clinical and radiologic follow-up analyses. Confirmation of a recurrence-free status was based on clinical and radiologic image analyses follow-up period of at least 12 months with no evidence of active malignancy. When a patient with at least one positive or equivocal study was confirmed to have recurrence, the FDG-PET/CT study was defined as true positive. When a patient with at least one positive or equivocal study was proved to have no recurrence, the FDG-PET/CT study was defined as false positive. When a patient with negative studies was later confirmed to have recurrence, the FDG-PET/CT study was defined as false negative. When a patient with negative study was confirmed to have no recurrence, the FDG-PET/CT study was defined as true negative. Among the patients who present with lung nodules, differentiation between recurrent lung cancer and second primary lung cancer were conducted based on criteria of Martini and Melamed [11]. Patients with a site of increased FDG uptake, which is considered to be other diseases than recurrent lung cancer, underwent diagnostic procedure for the site. Diseases other than recurrent lung cancer are diagnosed by clinical course or serial radiologic imaging or biopsies if possible.

3. Results

3.1. Clinical outcome

The time interval between initial surgery and latest follow-up in the present series ranged from 11 to 229 months (median 44 months), and the time interval between FDG-PET/CT and latest follow-up ranged from 2 to 34 months (median 19 months). At the end of the follow-up period, recurrences were confirmed in 35 (15%) patients, while 206 patients (85%) had no evidence of recurrence. Recurrence after treatment was as follows: lung metastasis in 11 patients, pleuritis carcinomatosa in 5, surgical margin recurrence in 2, mediastinal lymph node recurrence in 11, brain metastasis in 2, bone metastasis in 3, liver metastasis in 1. Representative case is shown in Figs. 1 and 2.

3.2. Performance of FDG-PET/CT study in diagnosis of recurrence

PET/CT correctly diagnosed recurrence in 34 of 35 patients and gave true negative findings in 198 of 206 patients who had no evidence of recurrence. Therefore, the performance indices of FDG-PET/CT for diagnosis of

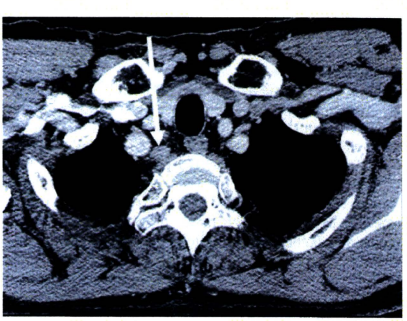

Fig. 1. A 57-year-old man with mediastinal lymph node recurrence after potentially curative resection of adenocarcinoma. Contrast-enhanced CT performed 10 months after surgical resection showd a right upper paratracheal node (arrow). The short axis diameter was 8 mm. CT, computed tomography.

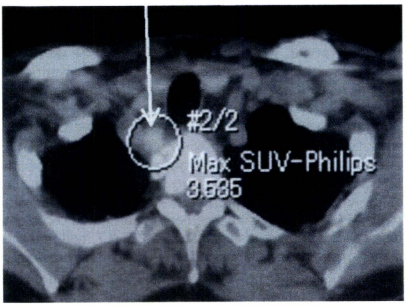

Fig. 2. FDG-PET/CT of the patient shown in Fig. 1 performed during the same period demonstrated high uptake of FDG (SUV_{max} = 3.5) within the right upper paratracheal node (arrow). This site was interpreted malignant and this FDG-PET/CT study was interpreted as positive. Radiologic follow-up analyses revealed that this case was true positive for the FDG-PET/CT study and radiation therapy was performed. FDG-PET/CT, F18-fluorodeoxyglucose positron emission tomography-computed tomography; SUV, standard uptake value.

recurrence, including sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were 97%, 96%, 96%, 81%, and 99%, respectively.

Analysis of the diagnostic value of PET/CT according to the reason for conducting the imaging studies showed that recurrence was confirmed in 24 (31%) of 78 patients complicated with some unfavorable events, but in 11 patients (7%) of 163 patients whose FDG-PET/CT was performed as a routine medical checkup.

3.3. Detailed profiles of false positive and false negative FDG-PET/CT studies

In 199 patients whose FDG-PET/CT was negative for recurrence during a follow-up period of 12–33 months (median 19 months) after PET/CT examination, one patient was finally judged as false negative because recurrences were detected. Recurrence in the patient was pleuritis carcinomatosa.

On the other hand, there were eight patients with false positive FDG-PET/CT studies. In four of these patients, the final diagnosis was postoperative change or benign inflammatory process in the thoracic cavity. Two patients were finally diagnosed with non-specific FDG uptake in distant organs. Two patients were finally diagnosed with other diseases (multiple myeloma and desmoid tumor of the chest wall).

3.4. Detection of other diseases by FDG-PET/CT studies

PET/CT studies detected 50 diseases other than recurrent lung cancer in 45 of 241 patients (19%). Twelve patients underwent treatment for the disease, which is detected by PET/CT studies. In nine patients (4%), malignancies were detected. Second primary lung cancer was detected in five patients. Four of these five patients underwent potentially curative surgery and one patient underwent definitive chemoradiotherapy. Malignancies other than lung cancer detected are as follows: one gastric cancer which was subsequently resected, one thyroid cancer which was resected, one pancreatic cancer which was treated by chemotherapy, and one multiple myeloma which was treated by chemotherapy. Two benign tumors detected by PET/CT were resected, one desmoid tumor of the chest wall and one teratoma of ovary.

4. Discussion

The present study retrospectively examined the diagnostic performance of FDG-PET/CT for recurrence in large-scale patients with NSCLC after potentially curative surgery. The results [sensitivity (97%), specificity (96%), and NPV (99%)] of FDG-PET/CT for recurrence demonstrated in the present study are as high as those in earlier studies summarized in Table 3 [6–10, 12, 13]. Although the tested patients were included not only for some reasons of recurrence-suspected events but also for medical routine checkup, the high diagnostic performance was obtained regardless of the reason for the examination. The PPV (81%) demonstrated in the present study is relatively low compared with earlier studies because of the false positive FDG-PET/CTs in our study discussed below.

Several groups reported that FDG-PET accurately differentiate recurrent cancer from benign inflammatory process in patients with clinically suspected recurrence [7, 9]. However, previous studies have some limitations, such as inclusion of patients with residual tumors after palliative treatment or patients with small cell lung cancer, or limited number of patients. The present study included the largest

Table 3
Comparison of results of previous studies on evaluation of recurrence of lung cancer using FDG-PET and FDG-PET/CT

Author	Year	Modality	Number of patients		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
			Total	After surgery				
Patz et al.	1994	PET	43	N.A.	97	100	100	89
Inoue et al.	1995	PET	38	15	100	62	84	100
Bury et al.	1999	PET	126	58*	100	92	92	100
Hicks et al.	2001	PET	63	63*	98	82	93	93
Keidar et al.	2004	PET/CT	42	40	96	82	89	93
Hellwig et al.	2006	PET	62	62	93	89	96	80
Takenaka et al.	2009	PET/CT	92	92	82	89	50	97
Present study	2009	PET/CT	241	241	97	96	81	99

*Including curative therapy without surgery, i.e., radiotherapy or chemoradiotherapy. N.A., not available; NPV, negative predictive value; PPV, positive predictive value; FDG-PET/CT, F18-fluorodeoxyglucose positron emission tomography-computed tomography.