plasia, respectively. CK 19 expression showed 10/10 and 5/5 for central and peripheral side metaplasia, respectively.

3.3. Staining scores of the C-type tumors and P-type tumors with each antibody

Table 5 shows the staining scores of the C-type tumors and P-type tumors with each antibody. CK 7 expression of C-type was little staining in almost all cases (Fig. 3a and c), whereas that of P-type was partial and moderate to weak positive in cancer nests (Fig. 3b and d). The average score of CK 7 was 10.9 ± 7.7 in the C-type and 43.2 ± 13.0 in the P-type, and difference was significant ($P = 0.0354$). Type II pneumocyte and bronchial glands were used as internal controls for CK 7 expression.

CK 19 immunoreactivity in the cancer nests in most C-type tumors was diffuse and strong (Fig. 3e and g), whereas staining in the P-type was partial and moderate-to-weakly positive (Fig. 3f and h). The average CK-19 score was 154.3 ± 18.0 in the C-type and 87.6 ± 16.9 in the P-type, and the difference was significant ($P = 0.0106$). A marginal difference in IGF-R and β-catenin immunoreactivity was detected. The difference in the E-cadherin scores was not significant but there was a significant difference in the rate. Staining for other cytokeratins, MUC family members, and for other antibodies showed insignificant immunoreactivity.

4. Discussion

In the current study, first of all, we investigated for clinical and pathological characteristics. It is difficult to explain why the patients with P-type SCC were older than the patients with C-type SCC. However, it may be related to mechanisms of carcinogenesis in each type of tumor. Furthermore, P-type SCC had higher vascular involvement and higher pleural invasion and with less lymph node metastasis compared with C-type SCC. This difference between C-type SCC and P-type SCC may be caused that C-type SCC often generates from bronchial membrane to inside of bronchi, on the other hand, P-type SCC exists in peripheral side of lung parenchyma. Although, P-type SCC showed higher vascular involvement, no difference in prognosis for overall survival in each type of tumor was shown (data not shown). This may be due to strictly case selection. Furthermore, in previous study at our institution, Funai et al. [4] examined the clinicopathological characteristics of peripheral squamous cell carcinoma of the lung, and they reports that C-type SCC showed a tendency to have N1 lymph node by direct invasion of tumor cells without lymphatic permeation, so P-type SCC had less lymph node metastasis compared with C-type SCC.

We have also reported the results of a clinicopathological study that efficiently used high-throughput TMA to evaluate differences in biological behavior between C-type SCC and P-type SCC of the lung. The most prominent examples of this cluster are cytokeratin 7 (CK 7) and cytokeratin 19 (CK 19). The expression patterns of these proteins were found to be different in C-type SCC and P-type SCC.

Cytokeratin, which is one of the five different types of intermediate filament is characteristically expressed in epithelial cells [18]. Cytokeratins are one of the main families of intermediate filaments, which make up the cytoskeleton. Cytokeratins are heterotypic tetramers of protofilaments composed of two polypeptides: one acidic type I subunit and one basic type II subunit [23]. Since each type of epithelium and its malignant counterpart express a specific cytokeratin pattern, the cytokeratin patterns of carcinomas are thought to be primarily determined by the cell type of origin and to be conserved throughout the multistage process of carcinogenesis.

CK 7 expression has been demonstrated in type II pneumocytes, peripheral bronchial epithelium, central bronchial epithelium, and bronchial glands in normal lung tissue [24]. Squamous metaplasia is also immunoreactive for CK 7 expression (data not shown). CK 7 expression was more predominant in P-type SCC than in C-type SCC. Broers et al. found that some SCCs are only focally immunoreactive for CK 7, because of tumor heterogeneity [25]. In a light microscopy study, McDowell et al. [16] observed that some SCCs differentiate into glandular epithelium. In our own study, P-type SCC was focally immunoreactive for CK 7, and a few PAS-Alb-positive cancer cells were scattered in P-type SCC, the same as in a previous review [17] suggesting that P-type SCC may have more characteristics of epithelial glandular cells than C-type SCC. Since CK 7 is well known to be strongly expresses in adenocarcinoma of the lung, P-type SCC may have some characteristics in common with adenocarcinoma [7,8].

CK 19 is a specific cytoskeletal structure of simple epithelium, including bronchial epithelium [18,19], and is strongly expressed by lung cancer tissue [25]. In our study, CK 19 stained positive in the type II pneumocytes, peripheral bronchial epithelium, central bronchial epithelium, and bronchial glands of normal lung tissue and in metaplastic epithelium. A previous study [13] reported abundant CK 19 expression in SCC of the lung. A novel tumor marker for measuring the CK 19 fragment, referred to as CYFRA 21-1, has recently been identified and proven accurate for diagnosing non-small cell lung cancer, particularly, SCC [20–22]. In SCC tissue, CK 19 expression was more predominant in C-type than in P-type SCC. Based on these findings and the fact that predominant squamous metaplasia was observed more frequently in C-type SCC, C-type SCC may arise from bronchial metaplastic epithelium, in agreement with previous reports [14,15,26,27]. The analysis of CK expression of squamous metaplasia has been sampled specifically, so there is a limitation in this analysis. CK19 expression was present in all of the layers of metaplastic epithelium on both the central side and the peripheral side, and CK 7 expression was almost always present in all layers of the metaplastic epithelium on both the central and the peripheral side. It is not explainable that C-type SCC is generated from bronchial metaplastic epithelium. We cannot explain why P-type SCC expressed CK 19 more weakly expression than C-type SCC, however the pathogenesis of P-type SCC have to be different from that of C-type SCC. The weaker CK 19 expression in P-type SCC may be due to different normal counterparts compared with that in C-type SCC. Alternatively, CK 19 expression might be downregulated by genetic and/or epigenetic alterations during tumor development in P-type SCC. The biological significance of this difference in expression pattern between these two types of SCC is not clear and merits further investigation.

In conclusion, we first demonstrated differences in cytokeratin expression pattern between C-type SCC and P-type SCC. CK 7 expression was more predominant in P-type SCC than in C-type SCC, whereas CK 19 expression was more predominant in C-type SCC than in P-type SCC. These results suggest that C-type and P-type SCC have different biological features.

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Review

Present Status of Clinical Proteomic Analysis for the Early Detection and Determination of Therapeutic Strategy in Lung Cancer

Takashi Hirano, MD, PhD and Harubumi Kato, MD, PhD

Although human DNA sequences have already been successfully decoded, there is still much we do not understand about the pathogenesis of human diseases. Proteomic analysis is comprehensive analysis of proteins, and there is a high probability that recent advances in this field will enable its application to individualized therapy based on thorough understanding of the pathogenesis of human diseases. Two-dimensional polyacrylamide gel electrophoresis (2-DE) and mass spectrometry (MS) are indispensable tools for proteomic analysis, and these technologies enable discovery of tiny changes of protein expression associated with pathogenesis. In this review we introduce the present status of proteomic analysis for cancer-related proteins, and especially lung cancer-related proteins in the attempt to discover new biomarkers for early detection and determine novel target-molecules for treatment. We believe that proteomic analysis will provide crucial information for diagnosis and treatment. (Ann Thorac Cardiovasc Surg 2006; 12: 4–9)

Key words: proteome, two-dimensional polyacrylamide gel electrophoresis, mass spectrometry, lung cancer

Introduction

"Proteome" and "proteomics" are relatively new words, coined by Wilkins et al. in 1996.[1] Proteomic analysis means comprehensive analysis of proteins, and proteomics is the science by which proteins are comprehensively investigated with regard to their roles as functional elements. The clarification of the human genome sequence is one of the most brilliant events in life science, and its results are accelerating comprehensive analysis of human gene products, "proteins". If the relationship between gene and protein were a one-to-one correspondence, many researchers might not recognize the importance of proteomic analysis. As we know, a gene alone is only potential information that must be put into a functional form, even though the pathogenesis of a malignant neoplasm is inherently associated with genetic disorders. The DNA is transcribed into RNA, then translated into protein. During this sequence, a number of alterations or modifications occurring at transcriptional, translational and post translational levels profoundly affect function. Put another way, several proteins with different functions may be derived from one gene through either alternative splicing at the RNA level or post-translational modifications at the protein level, for instance phosphorylation. It is well-known that phosphorylation is one of the most critical phenomena in cellular functions, and that it may be intimately associated with crucial events in the pathogenesis of some human diseases, for instance malignant neoplasm. Many researchers believe that proteomics will play a dominant role in life science in the post-genome age. Though recently "post genome" as a technical term is frequently used, it would be more precise to say that at present we are in the age of the post-genome sequence, and that we now stand at the entrance to the age of functional analysis at the molecular level. The main molecules carrying out physiological as well as pathological func-

From Department of Surgery, Tokyo Medical University, Tokyo, Japan

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Address reprint requests to Takashi Hirano, MD, PhD: Department of Surgery, Tokyo Medical University, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan.

tions are, of course, proteins. Therefore, it is necessary to investigate human proteins to understand the pathogenesis of human diseases.

Human genome analysis has shown that there are approximately 35,000 human genes, and it is assumed that more than 100,000 proteins must be expressed in the human body. In addition, it is important to understand the three-dimensional structures of protein molecule to understand their functions. Even though it is very complicated to analyze protein molecules, we cannot avoid investigating proteins for the complete elucidation of the pathogenesis of any human diseases. Therefore, new technology that combines simplicity, high through-put and automatic analysis is required. In this context, recent advanced proteomic technologies have brought the hope of discovering novel biomarkers that can be used to detect the early stage of disease, to predict the effectiveness of therapy and to monitor disease progression. The precise prediction of both therapeutic effects and adverse reactions must lead us to individualized therapy. In this review we discuss the present status of clinical proteomics for cancer-related proteins, and especially its application in the field of lung cancer is reviewed. We believe that understanding the present concepts of proteomic analysis in the field of oncology is extremely valuable for thoracic surgeons who will develop new therapeutic strategies for malignant neoplasms. The final purpose of clinical proteomics is to improve diagnostic procedures including the early detection and exact evaluation of the biological characteristics of diseases, and to understand the molecular pathogenesis of diseases to permit novel therapeutic strategies.

Commencement of Clinical Proteomics– Two-dimensional Polyacrylamide Gel Electrophoresis (2-DE) Based Strategies

Though the word "proteome" as a technical term is new, the concepts of comprehensive protein analysis have been established from 1975. At that time, O'Farrell established high resolutional two-dimensional polyacrylamide gel electrophoresis (2-DE) for comprehensive protein analysis.[2] In this method proteins are separated using isoelectric focusing (IEF) and sequential sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Approximately 1,000 proteins can be evaluated on 2-DE gel showing isoelectric point and molecular weight.

We started proteomic analysis using the 2-DE method on surgically resected materials of solid cancers in 1989. We initially used frozen surgically resected materials, but high resolutional analysis for cancer-related proteins was not possible due to contamination with serum proteins and necrotic substances as well as normal cells such as mesenchymal cells and inflammatory cells. Until we developed a new sample preparation method using fresh surgically resected materials, it was impossible to investigate cancer-related proteins with high resolution.[3] After the establishment of this non-enzymatic sample preparation technique we investigated the histopathological magnification of each histological type of primary lung cancer using clinical materials of primary lung cancer. More than 80% of proteins detected on 2-DE gels were not specific for any histological types. However, 2-DE patterns shown by the expression of several proteins reflected the histopathological differentiation of the primary lung cancer. During this investigation we identified 13 proteins associated with histopathological features. Most well-differentiated cases show high expression rates of proteins associated with the same histopathological differentiation and very low expression rates of proteins associated with other histopathological differentiation. On the other hand, in poorly differentiated cases we recognized a relatively wide variation of expression rates in the proteins associated with histopathological differentiation. In addition, when we evaluated primary lung adenocarcinoma, cases with the typical 2-DE pattern for lung adenocarcinoma showed a favorable outcome, and on the other hand the other cases showing the atypical 2-DE patterns showed a relatively poor outcome. The classification based upon the expression of cancer-related proteins may reflect biological characteristics of the tumor as well as histological differentiation.[4]

During the investigation of histopathological differentiation-related proteins, we detected one protein with high intensity only in primary lung adenocarcinoma, TA02. This protein molecule was not expressed in either metastatic lung adenocarcinoma from the other organs or the other types of primary lung cancer except a few cases of large cell lung cancer, and in normal human tissues this molecule was distributed only in type II pneumocyte and a part of the renal tubules.[5,6] At present, we understand that TA02 is homologous with napsin A, a new type of aspartic proteinase, which is involved in the maturation of the biologically active form of surfactant apoprotein B (SpB). It is suggested that Napsin A cleaves the N-terminal peptide of SpB, resulting in a 25 kDa intermediate.[7,8] We believe that our 2-DE investigation is the first report concerning proteomic analysis using clinical samples of

primary lung cancer.

Hanash and their colleagues at the University of Michigan Medical Center constructed a database that contained protein expression data on lung cancer based upon 2-DE findings. Also, they identified histopathology-related proteins. They described the possibility of developing novel classification schemes for lung cancer and the identification of novel markers for early detection using this kind of 2-DE protein database.[9] They also investigated 93 tissue samples of lung adenocarcinoma and 10 samples of normal lung tissues, and identified 9 protein molecules with significant overexpression in lung adenocarcinoma.[10] Furthermore, they detected 46 survival-associated proteins by 2-DE. Sequentially, 33 out of these 46 proteins were identified using MS, and among these candidate proteins, phosphoglycerate kinase 1 was validated as a survival-associated protein based upon another investigation of both tissues and serum derived from the patients with non-small cell lung.[11]

Proteome Platforms Not Involving 2-DE

Though 2-DE is really one of the most powerful tools for proteomic analysis, it does have several shortcomings. It is too intricate to permit automatic analysis, and its reproducibility is sometimes poor. Also, highly abundant proteins interfere with identification of less common proteins on 2-DE gel when either total-cell lysates or tissue lysates are applied to 2-DE analysis. When either cell lysates or tissues lysates are analyzed, approximately 10^6 orders in the dynamic range seem to be required. Furthermore, it is difficult to investigate extremely acidic or basic proteins and hydrophobic proteins, e.g. membrane proteins, which are strongly associated with cellular functions as a growth factor receptor. Therefore, new technology with high through-put and wide dynamic range as well as with high sensitivity have been eagerly awaited.

After bioinformatics made remarkable progress due to the completion of the human genome project, high through-put proteomic technology rapidly developed in the last 10 years. Finally, Fenn and Tanaka received the Nobel Prize for Chemistry in 2002, and proteomic analysis using mass spectrometry (MS) became the center of attention. They developed ionization technology, which is essential for MS. Tanaka developed matrix-assisted laser desorption/ionization (MALDI), and Fenn also developed electro-spray ionization (ESI), which are essential elements in MS instrumentation.

The US Food and Drug Administration (FDA) and National Cancer Institute in the US set up a Clinical Proteomics Program in 2001. The next year they reported that a specific serum-proteomic pattern of ovarian cancer was identified compared with patients and healthy donors. They used a surface-enhanced laser desorption/ionization (SELDI) MS system, which is an affinity-based MS method using a protein chip modified with a specific chromatographic surface. The SELDI MS system is a modified MALDI MS system, and it is based upon the principle that the proteins from crude mixtures are selectively attached to specific biochemical surfaces. Some proteins as potential biomarkers candidates may show a higher binding affinity to certain surfaces than common serum proteins. Serum samples from healthy donors and disease-affected individuals are processed using this protein chip. After washing steps, matrix is added to the protein spots and each proteomic pattern is acquired. Samples can be classified into a normal group, a disease-affected patients' group and additional groups, through the application of bioinformatic algorithms. SELDI MS analysis does not absolutely rely on the actual identification of the proteins to diagnose a disease. Therefore, this system has been used to detect some biomarkers (proteomic patterns) in complex protein mixtures such as cell lysates, body fluids and serum (Fig. 1).

According to initial reports concerning ovarian cancer by Petricoin et al., the discriminatory proteomic pattern correctly identified all 50 ovarian cancer cases, including 18 stage I cases. Of the 66 cases of non-malignant diseases, 63 were recognized as not being cancer. The sensitivity was 100%, specificity 95%, and positive predictive value 94%.[12] Furthermore, they investigated early detection of prostate cancer, and suggested that pathologic states within the prostate might be reflected by changes in serum proteomic patterns in relation to serum prostate specific antigen (PSA). The proteomic pattern correctly predicted 36 out of 38 patients with prostate cancer, while 177 out of 228 patients were correctly classified as having benign conditions. For men with marginally elevated PSA levels, the specificity was 71%. They concluded that serum pattern diagnostics might be of value in deciding whether to perform a biopsy on a man with an elevated PSA level.[13] This new approach is based upon the protein patterns analysis using SELDI and may provide a more effective means to diagnose some kinds of malignant neoplasm, such as ovarian, prostate, breast, and lung cancer. Over the past three years, many investigators have reported that pattern recognition algorithms based upon SELDI MS data may be successfully used to distinguish

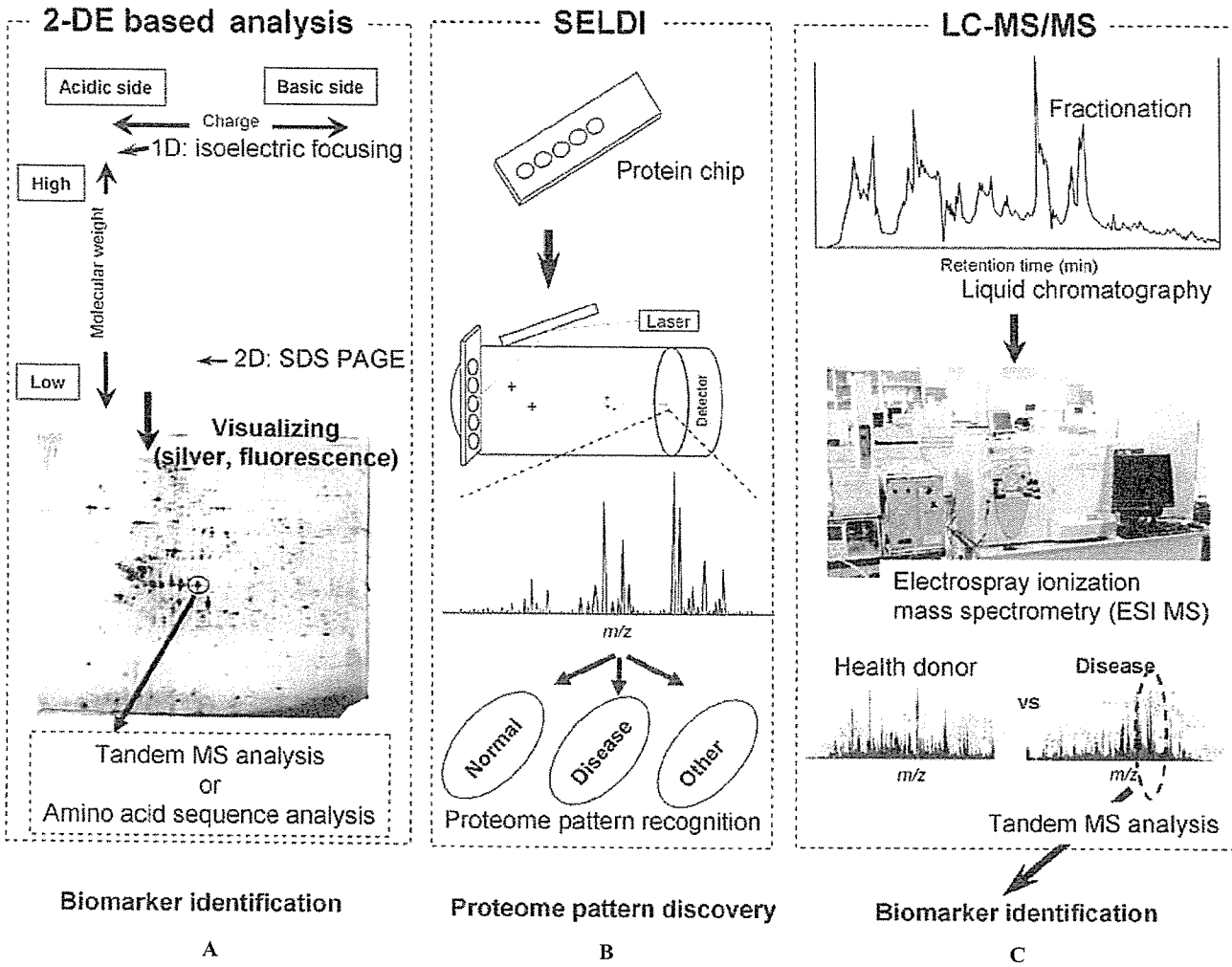

Fig. 1. Strategy for clinical proteomics.

A: Protein identification based upon two-dimensional polyacrylamide gel electrophoresis (2-DE).
A glass tube, to which the gel is cast, is used for isoelectric focusing (IEF). A sample is applied to each tube, and focused for approximately 15 hours. After the IEF gel is extruded into an equilibration buffer, sodium dodecyl sulphate (SDS) polyacrylamide gel (slab gel) is used for the second dimension. The IEF gel is sealed using agarose on the top of the slab gel, and is electrophoresed overnight. Subsequently, 2-DE proteins are visualized by either silver-staining or Comercy Blue staining.
After cutting the gel for extraction of protein, protein molecules are identified using tandem mass spectrometry (MS) or amino acid sequence analysis and bioinformatics.

B: Proteomic pattern discovery using surfaced enhanced laser desorption/ionization mass spectrometry (SELDI MS).
When the SELDI MS system is used for a diagnostic proteomics approach, samples from healthy donors and disease-affected individuals are processed using a protein chip modified with a specific chromatographic surface, and proteomic patterns are obtained as a result of the affinity to the surface of each protein chip. According to bioinformatics algorithms, the raw data are investigated to classify into either the healthy, disease, or other groups. This strategy does not rely on the actual identification of the protein molecules.

C: Protein identification using liquid chromatography-mass spectrometry (LC-MS).
This method relies upon (multidimensional) fractionation and tandem MS for protein-molecule identification. Samples derived from patients with specific diseases are compared with those derived from matched healthy donors. Attempts are made to discover unique or highly abundant proteins. Due to quantitative analysis of peptide-signal intensity and statistical analysis, it is possible to detect statistically significant differences in signal intensity, and the source protein molecule is identified using tandem MS analysis, which is performed sequentially.

between serums derived from normal donors and cancer patients.[14-17]

On the other hand, there are some reports criticizing proteomic pattern analysis using SELDI MS as not being reproducible or reliable enough for practical applications.[18,19] Finally, they concluded that the proteomic patterns that enable successful classification are biologically implausible and the methods, properly applied, do not classify the data accurately. Though commercial laboratories planned to market a test in late 2003 or early 2004, the US FDA in the US delayed starting clinical applications, because the question has not yet been resolved. We conclude that identification of a biomarker molecule is necessary when using serum biomarkers clinically, even though combination diagnosis with several kinds of biomarkers is undertaken.

Proteomic Analysis of Lung Cancer Using MS

There are few reports concerning proteomic analysis using MS for the investigation of clinical lung cancer materials. Yanagisawa et al. investigated proteomic patterns of non-small cell lung cancer using MALDI-time of flight MS (MALDI-TOF MS). They reported that class-prediction models completely classify histology, distinguish primary tumors from metastatic lesions from other organs to the lung, and classified nodal involvement with 85% accuracy. Also, they obtained a proteomic pattern comprised of 15 distinct MS peaks that distinguish between patients with poor prognosis and good prognosis.[20] However, they went no further than recognizing the proteome pattern. Therefore, the molecules related to specific events were not identified in this study.

Tyan investigated pleural effusion fluid derived from lung adenocarcinoma using two-dimensional liquid chromatography (LC) tandem MS, and it was reported that 124 proteins were identified. Based upon previous reports, it was concluded that 69 proteins among these proteins originated from plasma and that another 13 proteins were synthesized in the lung. Finally, a protein database concerning human pleural effusion will provide potential protein diagnostic biomarkers to be examined in further investigations.[21]

We also continue to make efforts to explore biomarkers related to lung cancer using LC-MS. Recently, we established a high-throughput comprehensive protein profiling system comprising a fully automated on-line microflow LC/tandem MS system for clinical sample utility. In this system quantitative evaluation of signal intensity analyzes statistically significant differences between two groups, for instance groups of healthy donors and cancer patients. Furthermore, automatic operation enabled the completion of a single run of entire LC-MS/MS analysis within 11 hours. Investigation of the data extracted from the protein identification datasets of both groups could allow identification of candidate proteins of disease-specific biomarkers. We applied this high throughput micro LC-MS/MS protein profiling system to surgically resected tissues and plasma derived from patients with primary lung adenocarcinoma, and attempted to identify the specific protein-molecules showing statistically significant differences in protein-expression levels. Firstly, we applied this protein profiling system to the investigation of the proteins associated with lymph node metastasis compared with expression profiles of two groups. One group consisted of cases without lymph node involvement, and the other group consisted of cases less than 3 cm in the largest dimension with lymph node involvement. The profiles were accumulated for each group using our originally developed profile alignment program. The statistical selection was done by Student's t test using a p-value of less than 0.005 as a cut-off value. The number of selective data points was 5,889. We have to note that the number of data points is not identical to the number of proteins. Generally, one protein produces several kinds of peptide molecular ions, and each signal is expected to correspond to a single LC-MS signal. Among 5,889 significant points, 2,753 associated with protein identification information were derived from MS/MS data by the MASCOT™ protein identification software. Finally, we identified more than 500 protein molecules as potential biomarkers associated with lymph node involvement. However, a validation process is needed for clinical application of these results.

We also started a project to mine novel biomarkers for the early detection of primary lung adenocarcinoma. We believe that such protein may leak from cancerous tissue but their concentration may be less than 100 femto mol. The lower limitation of the detectable range in LC-MS system is the same order as the concentration of tissue leakage proteins. Plasma proteins consist of a large amount of classical proteins including albumin, globulin, several kinds of complement and fibrinogen, and a very small amount of tissue leakage proteins and interleukins. We used serum albumin- and Immunoglobulin-depleted samples in plasma proteome analysis, and succeeded in identifying more than 100 proteins.[22] However, at present it is difficult to reliably detect a very small

amount of protein such as tissue-leakage proteins derived from malignant solid tumor. We believe that a multi-dimensional analysis system is needed before MS analysis for plasma biomarker discovery, which is the primary aim in clinical plasma proteome projects.

Conclusion

Though recent advances in proteomic analysis are conspicuous, nevertheless current research has not yet achieved the final clinical goal of producing specific biomarkers for the early detection and discovery of new molecular targets for individualized therapy. The target molecules to be detected in proteomic investigations, must be sufficiently abundant to be clinically useful. It is necessary to further improve our techniques concerning sensitivity and wide dynamic range sufficiently to analyze human plasma proteins. We believe that the remaining problems will be resolved within a few years, and that a fundamental revolution in both early detection and therapeutic strategy will occur in the near future.

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Surgery for Bronchioloalveolar Carcinoma and "Very Early" Adenocarcinoma: An Evolving Standard of Care?

Valerie W. Rusch, MD, Ryosuke Tsuchiya, MD, PhD,† Masahiro Tsuboi, MD,‡ Harvey I. Pass, MD,§ Dominique Grunenwald, MD,|| and Peter Goldstraw, FRCS¶*

Abstract: Lobectomy and mediastinal lymph node dissection is the standard surgical management of early stage non-small cell lung cancer (NSCLC) because more limited resections have been associated with a higher risk of local recurrence. Nevertheless, recent lung cancer screening studies have led to the detection of an increasing number of "very early" NSCLC (defined as less than 2 cm in size) and of good-prognosis histologic subtypes, bronchioloalveolar carcinoma (BAC), and adenocarcinoma (AC), mixed subtypes that are potentially appropriate for sublobar resection. The precise indications for sublobar resection remain unclear and are the subject of ongoing clinical trials, but it seems that very early, peripherally located, node-negative AC of a predominantly BAC pattern may be adequately treated in this manner. Multifocal AC and BAC, either synchronous or metachronous, are also effectively treated by complete resection, using limited resections whenever possible. The pneumonic form of BAC, the rarest variant of this disease spectrum, continues to have a poor prognosis despite complete resection. Very limited experience suggests that lung transplantation leads to prolonged survival in highly selected patients with this histologic subtype. To improve our management of very early AC, much more information is needed about the molecular abnormalities of AC and their relationship to clinical outcomes.

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During the past decade, thoracic surgeons have been confronted with demographic and pathological shifts in the group of non-small cell lung cancers (NSCLC) that are potentially resectable.[1] In many countries, adenocarcinoma (AC) has become the most common NSCLC histology. The proportion of women with lung cancer has increased dramatically; in some institutions, half of all patients are female. The number of patients who have never smoked or who have minimal past tobacco exposure is also increasing, especially in North America, because of tobacco control efforts. The widespread use of computed tomography (CT) for lung cancer screening has also led to increased detection of "very early" NSCLC, generally defined as tumors that are 2 cm or less in size, which are usually ACs of mixed subtype or bronchioloalveolar carcinomas (BAC) and which tend to have an indolent clinical behavior.

These epidemiologic shifts have led thoracic surgeons to reexamine the accepted tenets of surgical management of early-stage NSCLC. As part of the November 2004 symposium on BAC, which is the subject of this supplement, a group of thoracic surgeons were asked to review the current management of BAC and very early ACs, focusing especially on the role of sublobar resection. This paper summarizes the discussions held at the symposium and provides updated information on relevant clinical trials.

PATHOLOGICAL CLASSIFICATION OF AC: RELEVANCE TO SURGICAL MANAGEMENT

BAC has long been recognized as a distinct form of AC associated with a favorable prognosis. In 1989, the North American Lung Cancer Study Group (LCSG) reviewed 1635 patients who had undergone resection of AC, 235 of whom had BAC. Resectable BAC occurred more frequently in never-smokers, was diagnosed at an earlier disease stage, and was associated with a better survival rate than invasive AC.[2] During the last 40 years, improved understanding of the pathology of lung AC has prompted substantial changes in the histologic subclassification by the World Health Organization (WHO), which are summarized by Travis et al.[3] in their report from the pathology panel of this symposium (Table 1). From 1967 to 1999, multiple subcategories were added to reflect increasing knowledge about the histologic heterogeneity of AC. Significant changes in the 1999 WHO classification included the addition of atypical adenomatous hyperplasia (AAH) as a preinvasive lesion for lung AC, and the requirement that all BACs demonstrate pure lepidic growth without invasion of stroma, blood vessels, or pleura. In 2004, AC mixed subtype was moved to the top of the list of subcategories in recognition that this is now the most common subtype.[4]

In 1995, Noguchi proposed a six-tier histologic subclassification (types A through F) for small ACs of the lung, recognizing the excellent prognosis associated with BACs (with a purely lepidic growth pattern), the adverse prognostic

*Memorial Sloan-Kettering Cancer Center, New York, New York; †National Cancer Center Hospital, Tokyo, Japan; ‡Tokyo Medical University, Tokyo, Japan; §New York University Medical Center, New York, New York; ||Hôpital Tenon, Paris, France; and ¶Royal Brompton Hospital, London, United Kingdom.

Address for correspondence: Valerie W. Rusch, M.D., Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; E-mail: ruschv@mskcc.org

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TABLE 1. History of Lung Adenocarcinoma Subclassification According to the World Health Organization

1967	Bronchogenic
	Acinar
	Papillary
	Bronchioloalveolar
1981	Acinar adenocarcinoma
	Papillary adenocarcinoma
	Bronchioloalveolar carcinoma
	Solid carcinoma with mucus formation
1999	Acinar
	Papillary
	Bronchioloalveolar carcinoma
	Nonmucinous
	Mucinous
	Mixed mucinous and nonmucinous
	Solid adenocarcinoma with mucin
	Adenocarcinoma with mixed subtypes
	Variants
	Well-differentiated fetal adenocarcinoma
	Mucinous (colloid) adenocarcinoma
	Mucinous cystadenocarcinoma
	Signet-ring adenocarcinoma
	Clear-cell adenocarcinoma
2004	Adenocarcinoma, mixed subtype
	Acinar adenocarcinoma
	Papillary adenocarcinoma
	Bronchioloalveolar carcinoma
	Nonmucinous
	Mucinous
	Mixed nonmucinous and mucinous or indeterminate
	Solid adenocarcinoma with mucin production
	Fetal adenocarcinoma
	Mucinous (colloid) adenocarcinoma
	Mucinous cystadenocarcinoma
	Signet-ring adenocarcinoma
	Clear-cell adenocarcinoma

From Travis WD, Garg K, Franklin WA, et al. Evolving concepts in the pathology and CT imaging of lung adenocarcinoma and bronchioloalveolar carcinoma. J Clin Oncol 2005;23:3279–3287. Used with permission.

TABLE 2. Noguchi's Histology Typing of Small Adenocarcinoma of the Lung

Type	Description
A	Localized bronchioloalveolar carcinoma
B	Localized bronchioloalveolar carcinoma with foci of collapse of alveolar structure
C	Localized bronchioloalveolar carcinoma with foci of active fibroblastic proliferation
D	Poorly differentiated adenocarcinoma
E	Tubular adenocarcinoma
F	Papillary adenocarcinoma with compressive and destructive growth

From Noguchi M, Morikawa A, Kawasaki M, et al. Small adenocarcinoma of the lung. Histologic characteristics and prognosis. Cancer 1995;75:2844–2852. Used with permission.

importance of central fibrosis in BACs, and the pathologic heterogeneity of invasive ACs (Table 2).[5] Although the 2004 WHO classification is the internationally accepted system, Noguchi deserves credit for an early attempt to refine the classification and to correlate it with clinical outcomes. As discussed below, the Noguchi system is still used by Japanese investigators to select patients for sublobar resection in ongoing clinical trials. More recently, Noguchi showed that these histologic subtypes have corresponding molecular abnormalities.[6] Areas of histologic types A, B, and C extracted by microdissection from resected ACs were examined by multiplex PCR-LOH and were found to have a progressive rise in the incidence of allelic losses. Deletions of 3p, 17p, 18q, and 22q increased significantly from types A to C, consistent with a model of malignant progression.

Several Japanese studies now confirm that the histologic subtype correlates with CT findings and clinical outcome.[3,7,19] The results of Kodama exemplify these investigations (Table 3). Taken as a whole, these studies suggest that: 1) pure ground-glass opacities (GGO) on CT usually represent BAC without any areas of invasive AC, whereas lesions that show both GGO and solid components on CT (part solid, part nonsolid) are mixtures of BAC and invasive ACs; and 2) small (less than 2 cm in size) tumors with >50% GGO are associated with a 100% chance of being node negative, have an excellent chance of long-term survival after treatment, and probably can be managed by limited resection rather than lobectomy. However, the appropriateness of limited resection for part solid/part nonsolid lesions is unclear and is the subject of clinical trials in Japan. Tumors that are more than 50% GGO on CT seem to have a better prognosis and may potentially be managed by sublobar resection, but preoperative high-resolution CT and intraoperative frozen-section analysis still do not always accurately identify tumors that have a poorer prognosis. Our uncertainties with respect to the optimal surgical management of these lesions reflect the highly variable presentation and behavior of lung ACs, the limitations of CT findings in predicting pathologic findings, and our lack of knowledge of the histologic and molecular features that predict a poor prognosis.

TABLE 3. Prognosis in Relationship to Appearance (% GGO)

	GGO < 50%	GGO > 50%	*p*
Patients	52	52	—
Size	13.7	12.3	0.09
Node involvement	8	0	0.01
% local resection	50%	70%	0.001
Relapse	9	0	—
DFS	72%	100%	—

GGO, ground-glass opacity; DFS, disease-free survival.

Adapted from Kodama K, Higashiyama M, Yokouchi H, . Prognostic value of ground-glass opacity found in small lung adenocarcinoma on high-resolution CT scanning. Lung Cancer 2001;33:17–25. Used with permission.

RELATIONSHIP OF TUMOR SIZE TO TUMOR STAGE: SURGICAL IMPLICATIONS

In NSCLC, the size of the primary tumor is known to correlate with the likelihood of lymph node metastases and, therefore, to influence consideration of sublobar resection. The frequency of nodal disease in very early NSCLC has been studied extensively.[20 32] Although lymph node involvement is relatively uncommon in small AC, approximately 10% of tumors that are 1 cm or smaller and 20% of tumors that are 1 to 2 cm in size have nodal metastases (Tables 4 and 5). Relative to AC, squamous cell carcinomas less than 2 cm in size seem to be associated with a lower risk of nodal disease.[20] These findings complicate the selection of patients for limited pulmonary resection because we do not fully understand which patients with very early lung AC may have disease in the intralobar lymphatics or regional nodes. A better understanding of the molecular features in early AC and their relationship to clinical outcome is needed to allow accurate decisions about the use of sublobar resection.

LOBECTOMY VERSUS SUBLOBAR RESECTION: CURRENT KNOWLEDGE AND INVESTIGATIONS

A prospective randomized multicenter trial reported by the LCSG in 1995 established lobectomy as the standard approach to resection for T1N0 NSCLC (LCSG trial 821). Sublobar resection, either wedge resection or segmentectomy, for carefully selected patients who had thorough intraoperative evaluation of the extent of the primary tumor and of the N1 and N2 lymph nodes, was associated with a tripling of the local recurrence rate and a 30% increase in the overall death rate. Within the T1 stage category, tumor size did not seem to influence the risk of recurrence, but the numbers of patients who had tumors less than 2 cm in size were small.[33] The increasing incidence of very early NSCLC seen in thoracic surgical practice, primarily via CT screening for lung cancer,[1] has reopened the debate about the use of sublobar resection. This debate is especially relevant to BAC and to some AC of mixed subtype because of their indolent clinical behavior and known propensity for multifocality. Patients with these AC histologic subtypes often have synchronous or metachronous primary tumors that are best managed by resection. Preservation of lung function through the proper use of limited resection can be a critical aspect of achieving prolonged survival and maintaining patients' functional capacity.[34 36] Several retrospective studies and prospective clinical trials suggest that the sublobar resection may be an appropriate operation for very early AC.[11,13,37 40] The parameters that currently seem to allow proper selection of patients for limited resection include tumor size (less than 2 cm and especially 1 cm or less) in combination with tumor histology (BAC or AC, mixed subtype with 50% or greater BAC component or AC, Noguchi types A or B), peripheral tumor location, and absence of N1 or N2 disease based on thorough intraoperative staging. The presence of GGO or of part solid, part nonsolid appearance on CT reflects these tumor characteristics. In ways that are not yet fully understood (aside from the presence of EGFR mutations in some tumors), these clinical and pathologic features represent tumors that most likely have an indolent biological behavior. The adequacy of wedge resection versus anatomical resection via segmentectomy remains undefined, although segmentectomy has been favored in Japanese studies because it provides an optimal deep margin of resection and removes the local lymphatic bed associated with the primary tumor.[39]

Japanese investigators have sought to confirm these selection criteria for sublobar resection through prospective multicenter clinical trials. JCOG trial 0201 (Figure 1), reported at the 2006 meeting of the American Society of Clinical Oncology (ASCO), enrolled patients with clinical

TABLE 4. Prevalence of Nodal Disease in Solid Nodules <2 cm in Size

	n	% Positive Nodes	% N2
Naruke (1993)[23]	287	40	50
Asamura (1996)[24]	174	20	60
Konaka (1998)[25]	171	17.5	66
Takizawa (1998)[26]	157	17	NS
Sugi (1998)[27]	115	19	66
Wu (2001)[28]	136	22	NS
Okada (2003)[29]	265	18	55
Nonaka (2003)[30]	46	28	70
Average		23	

NS. not stated.

TABLE 5. Prevalence of Nodal Disease in Solid Nodules 1 cm or Less in Size

	n	Patients with Positive Nodes (%)
Naruke (1993)[23]	20	8 (16)
Oda (1998)[31]	22	0 (0)
Konaka (1998)[25]	19	0 (0)
Ohta (2001)[20]	11	4 (4)
Miller (2002)[32]	100	7 (7)
Average		9

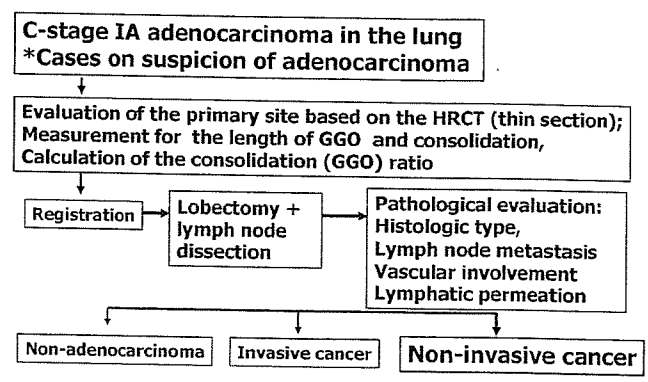

FIGURE 1. Schema for JCOG 0201 trial.

stage IA AC.[41] The primary endpoint was to determine the specificity of high-resolution CT (HRCT) in diagnosing noninvasive AC, using the final pathologic findings as the reference standard. A pathological noninvasive AC was defined as a tumor with no lymph node metastases or lymphatic or vascular invasion. Preoperative evaluation included HRCT to assess the presence of GGO and to calculate the ratio of GGO to solid component of the tumor. Patients then underwent lobectomy and mediastinal lymph node dissection. Final pathological findings were compared with the HRCT features to determine whether the CT could be used to select patients appropriately for sublobar resection. Of the 811 patients enrolled, 545 eligible patients had undergone lobectomy and central data review at the time of the ASCO presentation. Comparison of the CT with the pathological findings showed that HRCT had a specificity of 98.3% but a sensitivity of only 24.7% for the diagnosis of noninvasive AC.

The results of JCOG 0201 have been utilized to develop two new prospective trials. Patients found to have AC 2cm or less in size that are predominantly GGO by HRCT (solid component less than 25% of entire tumor) will be entered on a single arm Phase II trial testing the use of wedge resection for these highly curable indolent tumors. Patients found to have AC 2cm or less in size that have a larger solid component on HRCT (more than 25% but less than 100% of the entire tumor) will be eligible for a prospective randomized comparing lobectomy to limited resection (Figure 2). These trials might also help define which tumors do not require lymph node dissection or sampling, although this is not a planned study endpoint. At the current time lymph node sampling or systematic nodal dissection (SND) remains a key part of accurate tumor staging.[42]

In North America, the Cancer and Leukemia Group B (CALGB), in collaboration with the American College of Surgeons Oncology Group (ACOSOG), is planning a prospective randomized trial comparing lobectomy versus limited resection (wedge or segmentectomy) for patients with AC 2 cm or less in size. This trial does not incorporate the nuanced radiological and histologic selection criteria used in Japanese studies, depending instead on simple size criteria and the basic diagnosis of AC. Designed to reproduce the LCSG 821 trial, but with a focus on smaller tumors, the CALGB trial uses intraoperative assessment of tumor size, tumor location, and nodal involvement, followed by randomization to lobectomy or limited resection. Because of the large numbers of patients and long follow-up time required to identify a survival difference between these two resectional approaches, results from this trial will probably not be available for about 8 years.

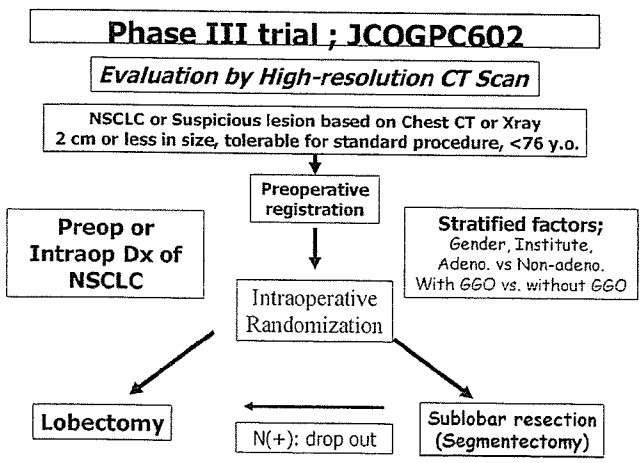

FIGURE 2. Schema for JCOGPC 602 trial.

MANAGEMENT OF THE PNEUMONIC FORM OF BAC: RESECTION, SYSTEMIC THERAPY, OR TRANSPLANTATION?

Most BAC or AC, mixed subtype present as either a single nodule or as multiple lung nodules (synchronous or metachronous) that behave in an indolent manner and are best managed surgically.[34,36,43] The least common variant of this BAC-AC disease spectrum is generally termed the pneumonic form because it presents as a progressive lobar consolidation with mucinous AC filling the alveolar spaces. Resection does not seem to alter the very poor prognosis of this disease, which inevitably progresses to consolidation of both lungs and death from respiratory failure.[34,43] Systemic therapy has also been relatively ineffective in this disease. Thus, most surgeons are reluctant to consider pulmonary resection for this biologically aggressive form of AC. Lung transplantation has been suggested as a potential treatment option. First reported by Zorn et al., lung transplantation in nine patients (single lung in two and bilateral transplants in seven patients) was associated with a poor outcome.[44,45] Only two patients survived long term, whereas the other patients experienced cancer recurrence in the transplanted lungs. More recently, the Toronto group reported their experience with transplantation in 29 patients.[46] Five-year survival was 51%, and recurrence developed in 13 of the transplanted lungs. Although transplantation was performed for advanced multifocal BAC, it is not entirely clear how many of these patients truly had the pneumonic form of mucinous AC. Thus, lung transplantation potentially remains an option for selected patients, but it is associated with a significant risk of recurrent disease and requires further study.

SUMMARY

Lobectomy and lymph node sampling or systematic nodal dissection remain the standard surgical treatment for patients with early stage NSCLC. However, limited resection may be an appropriate option for patients with very early AC and BAC based on tumor size, location, and relative proportion of BAC to AC. Very small BAC are probably appropriately treated by limited resection. Accurate criteria for selecting patients for limited pulmonary resection await the results of ongoing clinical trials and an improved understanding of NSCLC biology in relationship to clinical outcome.

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REVIEW ARTICLE

Tatsuo Ohira · Yasuhiro Suga · Yoshitaka Nagatsuka
Jitsuo Usuda · Masahiro Tsuboi · Takashi Hirano
Norihiko Ikeda · Harubumi Kato

Early-stage lung cancer: diagnosis and treatment

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Introduction

The lung cancer death rate is increasing throughout the world due to increases in numbers of the elderly, increased environmental pollution, and lack of detection in early stages. At our institution, the 5-year survival rate has gradually improved over the past five decades. These results could be due to improvements in therapeutic procedures, including surgery, chemotherapy, radiotherapy, laser therapy, and immunotherapy. Furthermore, the improvement in survival in Japan may be partially due to mass screening for lung cancer mandated by the Health Insurance Act of 1987. The therapeutic results for lung cancer are unsatisfactory. The 5-year survivals of lung cancer patients according to the Japanese Lung Cancer Registry, are shown in Fig. 1.[1] Good results were obtained only in stage I, but in other stages the results were still disappointing. Thus, in order to reduce deaths from lung cancer, it is necessary to detect and treat early-stage lung cancer.

However, there are various problems in the treatment of early-stage lung cancer. Early-stage lung cancers are classified into two categories according to the location of the tumor: central type and peripheral type, and the treatment of each type has specific problems.

In Japan, the criteria of early-stage lung cancer were first proposed about 30 years ago, in 1975. Peripheral-type early-stage lung cancer was defined as a tumor located in an airway more peripheral than the subsegmental bronchi, with the longest dimension of the tumor being 2 cm or less and with no recognized lymph node or distant metastases. In central-type early-stage lung cancer, the tumor is located in a segmental bronchus. In central-type lesions, even if they are early-stage lung cancer, resection of a large volume of lung is generally necessary. This could be a significant factor for pulmonary dysfunction, especially in older patients. In addition, lung cancer, especially the early-stage central type, has a tendency to develop in multiple lesions. In such cases resection is not a valid option for the treatment of all lesions. Therefore, noninvasive therapeutic modalities were required. Laser therapy has been developed for central-type early lung cancer. For the diagnosis of early-stage central-type lung cancer, autofluorescence fiberscopes, bronchofiberscopic echograms, and optical coherence tomography (OCT) have been developed.

As stated above, the improvement of survival in Japan may be partially due to mass lung cancer screening mandated by the Health Insurance Act of 1987. Mass screening for lung cancer by chest computed tomography (CT) was begun in Japan 10 years ago and is now being used in the United States and Europe. Because large numbers of tiny peripheral lung shadows were detected in many of the CT screening pilot trials,[2,3] it is important to establish an internationally accepted definition of peripheral-type early-stage lung cancer.

Therapeutic guidelines for central-type early-stage lung cancer

In Japan, the therapeutic guidelines for lung cancer were established according to evidence-based medicine, with the support of the Ministry of Health, Labor, and Welfare in 2002. In these guidelines, surgical resection and photodynamic therapy (PDT) are recommended for the treatment of central-type early-stage lung cancer.[4]

T. Ohira (✉) · Y. Suga · Y. Nagatsuka · J. Usuda · M. Tsuboi · T. Hirano · H. Kato
Department of Thoracic Surgery, Tokyo Medical University, 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
Tel. +81-3-3342-6111; Fax +81-3-3342-6154
e-mail: tatsuo@rd5.so_net.ne.jp

N. Ikeda
Department of Thoracic Surgery, International University of Health and Welfare, Tokyo, Japan

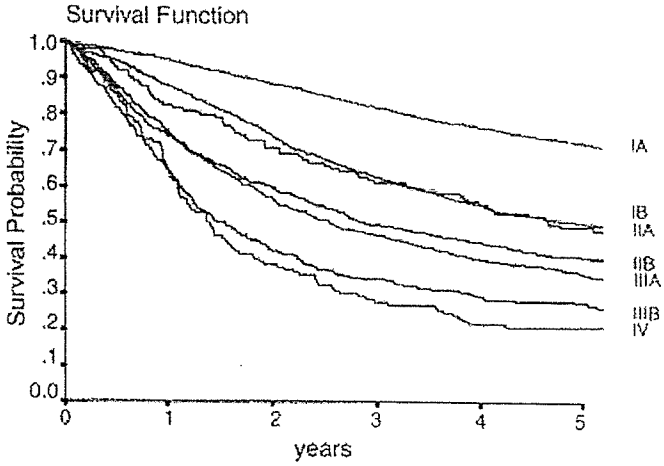

Fig. 1. Survival curves according to clinical (c)-stage. The 5-year survival rates according to c-stage were as follows: 72.1% for IA (n = 2423), 49.9% for IB (n = 1542), 48.7% for IIA (n = 150), 40.6% for IIB (n = 746), 35.8% for IIIA (n = 1270), 28.0% for IIIB (n = 366), and 20.8% for IV (n = 147). There was a significant difference in survival between stages IA and IB (P = 0.0000), between stages IIA and IIB (P = 0.0458), between stages IIB and IIIA (P = 0.0439), and between stages IIIA and IIIB (P = 0.0000). There was no difference in survival between stages IB and IIA (P = 0.4969) or between stages IIIB and IV (P = 0.1577).

Autofluorescence bronchoscopes (AFBs)

Central-type early-stage lung cancer can be cured by noninvasive endoscopic treatment, such as PDT, which has advantages for patients with poor pulmonary reserve; however, the detection of carcinoma in situ (CIS) is a challenge for bronchoscopists. Such lesions show only subtle changes in the bronchial mucosa,[5] and Woolner[6] reported that 60% of CIS lesions showed no macroscopically abnormal findings. This is particularly true with slightly edematous or superficial mucosal changes that can easily be missed, even by experienced bronchoscopists, because they are only a few millimeters thick. Autofluorescence diagnosis is a powerful method to detect macroscopically subtle lesions of the bronchus. Autofluorescence bronchoscopes (AFBs) have been used in leading facilities throughout the world, and the sensitivity for detection of intraepithelial lesions was reported to be 1.5 to 6 times higher than that of conventional white-light bronchoscopy.

Endobronchial ultrasonography (EBUS)

In order to decide indications for PDT, knowledge of the depth of the bronchial tumor is important. Previously, we assessed depth of tumor invasion by the shape of the tumor and loss of bronchus folds. Endobronchial ultrasonography (EBUS) can image the bronchial wall structure in order to assess the depth of bronchial tumor invasion.

Malignant tissues are imaged as hypoechoic areas, and tumor invasion of the cartilage layer is clearly detected. The bronchial wall structure can be imaged as six distinct layers. The cartilage layer is easily identified and can be used to evaluate bronchial wall invasion.

Optical coherence tomography (OCT)

Optical coherence tomography (OCT) is a new modality to detect early-stage lung cancer. OCT can obtain high-resolution, cross-sectional microscopic images of tissue, potentially enabling an optical biopsy to substitute for conventional excisional biopsy. We sought to investigate the capability of OCT to image the microstructure of normal and abnormal bronchial tissue. To assess the depth of bronchial tumor invasion, OCT imaging of the bronchial wall structure was clearer than EBUS, but OCT could detect only the surface of the bronchus.

The OCT system we used was produced by Light Lab Imaging (Boston, MA, USA) and Pentax (Tokyo, Japan). We inserted the OCT catheter via the working channel of the bronchoscope to evaluate the bronchial lumen. The catheter delivers a radial OCT beam and scans circumferentially to generate a transluminal image. In central-type lung cancers, the tumors showed unevenly distributed high backscattering areas and resultant loss of the normal layer structure. We believe that OCT will be able to detect nuclear structure and be used for diagnose is similarly to biopsy in the future.[7]

Possibility of limited resection by video-assisted thoracoscopic surgery (VATS)

The standard therapeutic procedure for peripheral-type early-stage lung cancer is believed to be lobectomy with mediastinal lymph node dissection. However the question has been raised whether lobectomy is really needed for tiny tumors, particularly those less than 1 cm in greatest dimension. There are several reports on limited resection of small lung cancers.[8,9] Some of these results showed satisfactory 5-year survival rates. Clinical trials to clarify the possibility of limited resection are needed for particularly small lung cancers showing ground-glass opacity (GGO), or ground-glass attenuation (GGA). Most of such lesions showed no lymph node metastases, and a 5-year survival of 100% was obtained in patients with such cases who underwent resection. Wedge resection of small lung cancers by VATS without lymph node dissection is one type of minimally invasive surgery. If some types of lung cancer could be shown to be resected by VATS without any increase in local recurrence, this method could become a future standard treatment for peripheral small lung cancers.

Rate of lymph node metastasis of peripheral small nodular cancers

In the past 5 years, 983 patients with lung cancer underwent surgery at our institution. Among them, a total of 159 pa-

tients were studied (Table 1). The tumor size was classified into three categories: 1 cm or less, 1 to 1.5 cm, and 1.5 to 2 cm (47, 49, and 63 patients, respectively). There were 147 pathological N0 patients; lymph node metastasis was recognized in 12 patients (7.5%); this was N1 in 3, and N2 in 9. Table 2 shows the rate of lymph node involvement according to tumor size. In patients with tumors of 1 cm or less, 98% showed no lymph node involvement; however, even in these tiny tumors, 2% showed N2 disease. In tumors between 1 and 1.5 cm, 94% showed no metastasis, but 6% were either N1 or N2. In tumors between 1.5 and 2 cm, lymph node involvement was recognized in 13%.

In this study, the percentages of GGO in tumors were extensively analyzed. We divided tumors into two categories according to how much of the lesion consisted of GGO findings. According to these criteria, 44 tumors showed more than 50% GGO and 115 showed less than 50% GGO. Tumors with a GGO ratio of more than 50% showed no lymph node metastases. On the contrary, all node-positive tumors showed a GGO ratio of less than 50% (Table 3). The relationship between percent GGO area on High Resolution Computed Tumography (HRCT) and the Noguchi classification[10] is shown in Table 4.

Twenty-five of the 44 tumors (57%) showing a GGO component of more than 50% on HRCT were Noguchi type A and B. Seventeen of the 71 tumors (24%) of type C showed more than 50% GGO, and the remaining 54 type C tumors (76%) showed less than 50% GGO. Fifty-three of the 55 (96%) type D, E, and F tumors showed less than 50% GGO. A good correlation between the CT findings and the Noguchi classification was recognized.

The relationship between representative clinicopathological factors and the percent GGO area is shown in Table 5. According to the χ^2 test, the percent GGO area was related to tumor size ($P = 0.0135$) and pathological stage ($P = 0.04$). In particular, a significant relationship with percent GGO was obtained for pathological features including the Noguchi classification ($P = 0.0001$), vascular invasion, and lymphatic invasion.

The overall 5-year survival rate of the patients studied was 88.0%, but it was 96.7% in those with tumors less than 1 cm in diameter, 81.6% in those with tumors between 1 and 1.5 cm, and 84.4% in those with tumors between 1.5 and 2 cm.

The 5-year survival rate was also analyzed according to percent GGO in the lesion. In patients with more than 50% GGO, a 100% 5-year survival rate was obtained, but those with less than 50% GGO had an 83.9% 5-year survival rate.

According to the Noguchi classification, a 5-year survival rate of 100% was obtained in types A and B, with 5-year survivals of 97.4% in type C, 67.1% in types D, E, and F, respectively, which was significantly lower than the results for types A and B and C.

Future surgical procedures for peripheral early-stage lung cancer

Tumors with 100% GGO findings on CT images could indicate suitability for limited surgical resection by VATS. Lesions showing between 50% and 100% GGO may also be indicated for limited resection in tumors less than 2 cm in

Table 1. Patient characteristics

Characteristics	
Age (years)	
Average	63
Minimum	40
Maximum	84
Sex	
Male	67
Female	92
Smoking habit	
Non-smoker	89
Smoker	70
Operative procedure	
Lobectomy	112
Segmentectomy	20
Wedge resection	27

Table 2. Tumor size and nodal status

Tumor size	N0	N1	N2
1.0 cm or less ($n = 47$)	46	0	1
1.0–1.5 cm ($n = 49$)	46	1	2
1.5–2.0 cm ($n = 63$)	55	2	6

Table 3. GGO area and TN status

GGO%	T ≦ 1 cm	1 < T ≦ 5 cm	1.5 < T ≦ 2 cm	
More than 50%	18	16	10	44
Less than or equal to 50%	29 (1)	33 (3)	53 (8)	115 (12)

Numbers in parentheses are numbers of node-positive tumors

Table 4. GGO area and Noguchi classification

GGO%	A, B	C	D, E, F	
More than 50%	25	17	2	44
Less than or equal to 50%	8	54	53	115

Table 5. Relationship between prognostic factors and percent GGO on HRCT

Prognostic factor	χ^2	P value
Sex	0.162	0.687
Tumor size	8.616	0.0135
Pathological stage: I or II–IV	4.168	0.0412
Noguchi classification: A, B, C or D, E, F	14.442	0.0001
Vascular invasion	6.76	0.0093
Lymphatic invasion	5.326	0.0206

diameter, and also, perhaps, in lesions showing between 10% and 50% GGO findings with a tumor size less than 1 cm in diameter. Evaluation of limited resection for small peripheral nodules was reported previously by several researchers.[8,9,11] However, different opinions concerning the modalities used have been reported.[12,13] There are still controversies concerning limited resection of peripheral small lung cancers. A randomized clinical trial by the Lung Cancer Study Group (LCSG) demonstrated the disadvantages of limited resection for T1N0 tumors in relation to lobectomy.[13] Therefore, clinical evidence of the usefulness of limited resection for peripheral early-stage lung cancers should be established. The features of peripheral lung cancers suitable for limited resection without lymph node dissection should be clarified. This will make it possible to determine the optimal CT findings for limited resection.

In our experience, even if the primary lesion was less than 1 cm in size, nodal involvement was confirmed histologically in some patients. Prognostic factors may depend not solely on tumor size but also on the percent GGO area. It is necessary to clarify the findings of CT images of noninvasive cancer by a clinical multicenter study.

Low-dose CT screening for lung cancer

Helical (spiral) CT imaging in the early 1990s provided a promising test for the detection of smaller nodules in the lungs, compared with traditional chest radiography, as images of the chest could be obtained in less than 20 s at a low dose of radiation. It is generally accepted that low-dose CT screening leads to early diagnosis of lung cancer in a high percentage of cases. Based on this evidence, annual CT screening provides for detecting the disease at earlier and presumably more commonly curable stages. The Early Lung Cancer Action Project (ELCAP) showed the great superiority of CT imaging over chest radiographic imaging in identifying cancerous "nodules" in the lungs.[14,15]

Adjuvant chemotherapy for early-stage lung cancer

Recently, some reports have shown significant survival results with adjuvant chemotherapy. The Japan Lung Cancer Research Group (JLCRG) conducted a randomized phase III trial of adjuvant chemotherapy with and without uracil plus tegafur (UFT) after complete surgical resection for stage I adenocarcinoma patients. Subgroup analysis of 263 stage IB patients showed a highly significant result for the UFT arm (5-year survival, 84.9% versus 73.5%; $P = 0.005$).[16]

Conclusions

Good results have been obtained in early-stage lung cancer treatments. Photodynamic therapy (PDT) is suitable for central-type early-stage lung cancer. VATS is a good indication for peripheral-type early lung cancer. Recently, less invasive therapies, such as stereotactic radiation therapy,[17] charged-particle therapy,[18] and microwave coagulation therapy[19] have shown promising results. PDT could be a good modality for peripheral lung cancer, too.[20] The important thing is to find the early-stage lung cancers.

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7192 General Poster Session (Board #U1), Sun, 8:00 AM - 12:00 PM

Correlation between epidermal growth factor receptor gene status and clinical outcome of gefitinib in Japanese patients with non-small cell lung cancer. *K. Kasahara, T. Sone, H. Kimura, K. Nishio, T. Tamura, K. Shibata, M. Mizuguchi, A. Yoshimoto, M. Fujimura, S. Nakao; Kanazawa University, Kanazawa, Japan; National Cancer Center Hospital, Tokyo, Japan; National Cancer Center Research Institute, Tokyo, Japan; Kouseiren Takaoka Hospital, Takaoka, Japan; Ishikawa Prefectural Central Hospital, Kanazawa, Japan*

Background: Epidermal growth factor receptor (EGFR) gene amplification and mutation have been reported to be predictors of response to EGFR inhibitors. We evaluated EGFR gene copy number and mutations in biopsy samples of non-small cell lung cancer (NSCLC) treated with gefitinib (G) and analyzed the correlation between gene status and clinical outcome. **Methods:** EGFR gene copy numbers in biopsy samples were evaluated using FISH and categorized as described by Cappuzzo et al. We also performed mutational analysesof exons 18, 19 and 21 of EGFR by PCR and direct sequencing. Response was judged using the RECIST guidelines. Time to progression (TTP) and overall survival (OS) were calculated and evaluated by the Kaplan-Meier method. Groups were compared using the log-rank test. Risk factors associated with survival were evaluated using Cox proportional hazards regression modeling and multivariable analysis. **Results:** Of 59 patients (pts) enrolled in this investigation, 24 pts (41%) were female and 21 pts (35%) had never smoked. The most common histological subtype was adenocarcinoma (73%) and 36 pts (61%) had good PS 0–1. Gene copy numbers could be analyzed in 54 pts. Gene amplification was observed in 21 pts, high polysomy in 5 pts, low polysomy in 18 pts, low trisomy in 5 pts and disomy in 5 pts. FISH positivity was observed in 26 pts (48%). EGFR mutations were detected in 18 pts (31%); point mutations in exon 21 were observed in 5 pts, in-frame deletions in exon 19 in 12 pts, a point mutation in exon 18 in 1 pt. Response rate in pts with EGFR mutations was significantly higher than in pts without mutations (56% vs. 15%, p = .0011). Response rate in FISH-positive pts was 31% and that in FISH-negative pts was 21%, the association with response was not significant. EGFR mutations were also correlated with improved TTP (median 8.3 m vs. 1.8 m, p = .0014) and OS (median 18.8 m vs. 6.4 m, p = .0059). There were no significant differences in TTP and OS based on FISH positivity. On multivariable analysis, EGFR mutations remained significantly associated with improved TTP and OS. **Conclusions:** Our results suggest that EGFR mutations are a better predictor of clinical benfit of G when compared with gene copy number in Japanese NSCLC pts.

7193 General Poster Session (Board #U2), Sun, 8:00 AM - 12:00 P

Prognostic significance of EGFR FISH and IHC in non small-cell lung canc patients treated with chemotherapy alone. *R. Dziadziuszko, B. Holm, B. Skov, K. Osterlind, W. A. Franklin, M. Varella-Garcia, P. A. Bunn Jr., F. Hirsch; University of Colorado Health Sciences Center, Aurora, CO; Herl University Hospital, Herlev, Denmark; Gentofte University Hospital, Copenhagen, Denmark; Copenhagen University Hospital, Copenhagen, Denma*

Background: High EGFR gene copy number by fluorescence in situ hybri ization (FISH) predicts response and survival benefit in non small-cell lu cancer (NSCLC) patients (pts) treated with EGFR tyrosine-kinase inhib tors, but its prognostic value remains debated. We aimed to evaluate t association of EGFR FISH, EGFR immunohistochemistry (IHC) and progn sis in NSCLC pts treated with chemotherapy alone. **Methods:** 85 pts treat with platinum-containing chemotherapy (median follow up of 15 mont [range: 2–29 months]) were included in the study. There were 47 females, 35% of pts with performance status (PS) 0, 53% PS = 1 and 12 PS = 2, 6% of never-smokers. Median age was 62 years (range: 41–8 years). Stage I-IIIA was diagnosed in 7%, stage IIIB - 44% and stage IV 48% pts. Adenocarcinoma was the most common histology (51% pts EGFR FISH was performed using LSI EGFR SpectrumOrange/CEP SpectrumGreen probe and IHC using DAKO PharmDx kit. **Results:** FIS results were available in 79 pts (93%), and EGFR FISH-positive tumc (high polysomy or gene amplification) were found in 28 pts (35%). IH results were available in 81 pts (95%) and 25 pts (31%) were scored positive (staining index ≥200). Distribution of clinical characteristics d not differ according to either FISH or IHC result. FISH-positive pts ha higher EGFR IHC staining indices as compared with FISH-negative p (median 160 vs. 60, p = 0.005, Mann-Whitney U test). Median survival FISH-positive pts was 12.6 months vs. 8.1 months in FISH-negative p (log-rank p = 0.68; HR = 0.88 [95% CI: 0.49–1.59]) and the respecti figures for progression-free survival (PFS) were 7.5 vs. 4.9 months, log-ra p = 0.72; HR = 0.91 [95% CI: 0.55–1.51]. Median survival in IH positive vs. IHC-negative pts was 6.6 months vs. 9.2 months (log-ra p = 0.44; HR = 1.27 [95% CI: 0.69–2.36]). There was no significa difference in PFS (median of 4.8 vs. 5.3 months, respectively; log-ra p = 0.71; HR = 1.11 [95% CI: 0.64–1.92]). FISH and IHC remain insignificant in a Cox regression survival analysis. **Conclusion:** In this cohc of NSCLC patients treated with chemotherapy alone, EGFR FISH w associated with EGFR IHC and both features had no statistically significa influence on prognosis.

7194 General Poster Session (Board #U3), Sun, 8:00 AM - 12:00 PM

Sorafenib combined with carboplatin/paclitaxel for advanced non-small cell lung cancer: A phase I subset analysis. *J. H. Schiller, K. T. Flaherty, M. Redlinger, K. Binger, J. Eun, O. Petrenciuc, P. O'Dwyer; University of Wisconsin, Madison, WI; University of Pennsylvania, Philadelphia, PA; Bayer, Inc., Toronto, ON, Canada*

Background: The EGFR is often overexpressed in advanced non-small-cell lung cancer (NSCLC) - a solid tumor associated with a poor prognosis. Oncogenic *k-ras* mutations and raised serum VEGF predict poor outcome in NSCLC. In vitro targets of sorafenib include Raf, which is downstream of EGFR and *k-ras*. Sorafenib also targets the VEGFR-2/-3 tyrosine kinases, involved in tumor angiogenesis. Preclinically, sorafenib targets the tumor and tumor endothelium to inhibit tumor growth. **Methods:** This subanalysis of a Phase I trial with a Phase II expansion in NSCLC was performed to evaluate the safety (adverse events graded by NCI-CTC 2.0) and preliminary anti-tumor activity (response by RECIST, PFS, TTP) of oral sorafenib combined with carboplatin/paclitaxel in 15 patients with advanced, progressive NSCLC. Carboplatin (AUC 6)/paclitaxel (225 mg/m^2) was administered on Day 1, and sorafenib (100, 200, or 400 mg bid) on Days 2–18 of each 21-day treatment cycle. **Results:** Drug-related adverse events were reported by 73% (11/15) of patients, but were mostly grade 1–2 (53%) in severity; none was grade 4. The most common drug-related events at any grade were dermatologic (Hand-foot skin reaction [20%]; rash [60%]), and gastrointestinal (diarrhea [20%]; anorexia [13%]). There were no drug-related cardiovascular adverse events. Three patients reported grade 1–2 drug-related bleeding events (epistaxis n = 2; other n = 1). Of the 14 evaluable patients, four (29%) had a confirmed PR as best response, seven (50%) had SD, and three (21%) had PD. Therefore, the disease control rate (objective response plus SD) was 79%. Duration of response was 25 weeks. Median PFS was 34 weeks. **Discussion:** This sorafenib combination was well tolerated and showed promising preliminary anti-tumor activity in patients with advanced, progressive NSCLC.

7195 General Poster Session (Board #U4), Sun, 8:00 AM - 12:00 P

Phase II open-label study to investigate efficacy and safety of PTK787/ 222584 orally administered once daily at 1,250 mg as second-line mon therapy in patients (pts) with stage IIIB or stage IV non-small cell lung canc (NSCLC). *T. C. Gauler, B. Fischer, J. Soria, V. Gounant, F. Griesinger, Krissel, D. Laurent, W. E. Eberhardt; West German Cancer Center, Esse Germany; University of Mainz, Mainz, Germany; Institut Gustave Rouss Villejuif Cedex, France; Hôpital Tenon, Paris, France; University of Goetti gen, Goettingen, Germany; Schering AG, Berlin, Germany*

Background: Overexpression of vascular endothelial growth factor recept (VEGF-R 1–3) in NSCLC is associated with poor prognosis and short overall survival. PTK787/ZK 222584 (PTK/ZK), a novel, oral, an angiogenic compound blocks tyrosine kinase signaling from all VEG receptors. **Methods:** A prospective, single-arm, multi-center, proof-c principle phase-II study to investigate both efficacy and safety of PTK/ Z in patients with stage IIIB/IV NSCLC who received prior first line treatme with a platinum based chemotherapy regimen. All pts were relapse refractory as determined by available imaging. All pts were planned receive 1,250 mg of PTK/ZK once daily (qd) for continuous treatment un disease progression or unacceptable toxicities. Response evaluation based on RECIST criteria. Disease stabilization of at least 12 weeks base on CT/MRI-imaging was defined as clinically relevant drug activity. All p had dynamic contrast enhanced MRI investigations for additional effica analysis and serum asservations for serum proteomic analysis. **Results:** T date, 56 pts have been enrolled. PTK/ZK is generally well tolerated. Mo frequent adverse events (AEs) are nausea and vomiting. One pt develope interstitial lung disease (ILD) and in one pt with tracheal stent a fatal lur bleeding occurred. No other unexpected serious AEs have been reporte Maximum response for 48 evaluated pts include one pt with a parti response (PR) (2%), 27 pts with stable disease (SD) (56%), (diseas control rate 58%), and 20 pts with progressive disease (PD) (42%). The with PR has responded for over 20 weeks. 15 pts have SD for at least 1 weeks; 5 pts of these had SD for at least 28 weeks. **Conclusions:** Thes preliminary results suggest that second-line treatment with single-age PTK/ZK is generally safe and well tolerated. In pts with refractory disease stage IIIB/IV NSCLC, a considerably high rate of disease control could b achieved in this study. The study is currently recruiting for twice daily (bi treatment.

7196 General Poster Session (Board #U5), Sun, 8:00 AM - 12:00 PM

Prognostic value of blood levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in advanced non-small cell lung cancer (NSCLC) patients. *V. Alberola, C. Camps, R. Sirera, L. Llobat, A. Blasco, M. J. Safont, J. Garde, M. Taron, J. J. Sanchez, R. Rosell; Hospital Arnau de Vilanova, Valencia, Spain; Hospital General de Valencia, Valencia, Spain; Hospital Germans Trias i Pujol, Badalona, Spain; Universidad Autonoma de Madrid, Madrid, Spain*

Background: VEGF and bFGF are among the most important angiogenic factors. We have explored these angiogenesis mediators in plasma and its prognostic significance in advanced NSCLC. **Methods:** Were enrolled 451 patients with advanced NSCLC, stages IIIB and IV and treated with cisplatin and docetaxel. Blood was collected before chemotherapy. Plasma VEGF and bFGF levels were assessed by commercial ELISA (sensitivity 5 pg/ml). In parallel plasma from 32 age and gender-matched controls was used. **Results:** Median age was 61 years (35–82) and 84% were males. 99% had performance status 0–1. 84% were in stage IV and 16% in stage IIIB. The histological subtypes were: 32% squamous cell carcinoma, 50% adenocarcinoma, 14% anaplastic large cell, and 4% undifferentiated. 41% of the patients received second line chemotherapy. 1% achieved complete response (CR), 36% partial response (PR), 35% had stable disease (SD) and 28% progressive disease (PD). Patient's median plasma levels of VEGF (20 pg/ml, [6–203]) differ significantly ($p = 0.04$) from controls (14 pg/ml, [7–53]), but in contrast bFGF levels were not different, 14 pg/ml [5–960] vs 10 pg/ml [6–278] respectively. There were not differences in patients according to histology, site of metastasis and ECOG; however we could observe a tendency with stage for both factors: bFGF 9 pg/ml [5–24] in stage IIIB vs 15 pg/ml [6–960], $p = 0.071$ and VEGF 17 pg/ml [6–145] in IIIB vs 21 pg/ml [6–203] in IV, $p = 0.086$. It could not be observed any differences in response to therapy for both angiogenic factors; CR+PR patients presented median VEGF of 18 pg/ml [6–71] and bFGF 11 pg/ml [6–960] vs 20 pg/ml of VEGF [6–203] and 15 pg/ml of bFGF [5–395] in the SD+PD group. In the multivariate analysis we could not find that VEGF and bFGF plasma levels were predictors for time to progression (TTP) and overall survival (OS). **Conclusions:** VEGF but not bFGF levels in patients are significantly higher in patients than in controls. In our cohort of patients with advanced NSCLC we have not found any relationship between serum VEGF and bFGF levels with stage, histology, response, site of metastasis, TTP and OS.

7197 General Poster Session (Board #U6), Sun, 8:00 AM - 12:00 PM

The influence of tumor size, histological differentiation and smoking history in patients with completely resected stage I adenocarcinoma of the lung. *M. Tsuboi, H. Kato, Y. Ichinose, M. Ohta, E. Hata, N. Tsubota, H. Tada, H. Wada, N. Hamajima, M. Ohta, the Japan Lung Cancer Research Group on Postsurgic; Tokyo Medical University, Tokyo, Japan; National Kyushu Cancer Center, Fukuoka, Japan; National Okinawa Hospital, Okinawa, Japan; Mitsui Memorial Hospital, Tokyo, Japan; Hyogo Medical Center, Akashi, Japan; Osaka City General Hospital, Osaka, Japan; Kyoto University, Kyoto, Japan; Nagoya University, Nagoya, Japan*

Background: To test the hypothesis that patients with completely resected p-stage I adenocarcinoma [Ad.] of the lung contain a favorable subgroup of patients with well differentiated histology and tumor 2.0 cm or less in greatest dimension, we analyzed the results of the JLCRG trial (a randomized prospective trial of adjuvant chemotherapy with Uracil-Tegaful for stage I adenocarcinoma of the lung) by tumor size, smoking history, degree of histological differentiation and more. **Methods:** Patients were randomized to receive either oral uracil-tegaful (250 mg of tegaful /m^2/day) for 2 years postoperatively or no adjuvant treatment. Multivariate analyses and interactions with the Cox proportional-hazards model were used to estimate the simultaneous effects of prognostic factors on survival. **Results:** The 5-year survival rate of the 412 patients with tumor 2cm or less in size was 89.8% (95% confidence interval [CI]: 86.8 to 92.8) versus 84.4% (95% CI: 81.3–87.4) for the 569 patients with tumor more than 2cm in size (median follow-up 72 months, $p = 0.002$). Although univariate analysis demonstrated improved survival for the patients with no smoking history and female gender, the selected covariates by multivariate analysis were as follows: age (hazard ratio [HR] for patients aged 70 years or more, 2.25; 95% CI: 1.58 to 3.14, $p < 0.0001$), tumor size (HR for more than 2cm in size, 1.55; 95% CI: 1.10 to 2.21, $p = 0.012$), histological differentiation (HR for moderate and poor differentiation, 1.75, 95% CI: 1.25 to 2.47, $p = 0.001$), and treatment group (HR for the uracil-tegaful group, 0.68; 95% CI: 0.49 to 0.94, $p = 0.02$). For these prognostic factors, there was only one significant interaction between tumor size and the adjuvant treatment. **Conclusions:** 1) Patients with completely resected stage I Ad. of the lung contain a favorable subgroup of patients with aged less than 70 years, well differentiated histology, and a maximum tumor dimension of 2.0 cm or less. 2) Adjuvant chemotherapy with oral uracil-tegaful should also be considered for stage I Ad. patients more than 2 cm in tumor size. 3) 2cm in tumor size might be a good benchmark candidate of the description of T factor to facilitate treatment strategies and revisions of the TNM staging system.

7198 General Poster Session (Board #U7), Sun, 8:00 AM - 12:00 PM

Elevated osteopontin (OPN) plasma levels are highly prognostic in advanced non-small cell lung cancer (NSCLC): Analysis of SWOG S0003. *P. C. Mack, M. W. Redman, K. Chansky, S. K. Williamson, N. Farneth, P. N. Lara Jr, Q. Le, P. H. Gumerlock, J. J. Crowley, D. R. Gandara; UC Davis Cancer Center, Sacramento, CA; Southwest Oncology Group Statistical Center, Seattle, WA; SWOG Statistical Center, Seattle, WA; University of Kansas Medical Center, Kansas City, KS; Stanford University, Stanford, CA*

Background: OPN is a secreted glycoprotein with a diverse array of functions, including induction of uPA & increased cell migration. OPN has been shown to be elevated in a number of tumor types, & its downregulation reduces tumorigenicity & metastasis in tumor models. High levels have also been associated with tumor hypoxia/angiogenesis, as are vascular endothelial growth factor (VEGF) & plasminogen activator inhibitor (PAI-1). We hypothesized that secreted levels of these biomarkers would correlate with clinical outcome after treatment. **Methods:** Plasma concentrations of OPN, VEGF & PAI-1 were measured by ELISA in 160 NSCLC patients enrolled on the Southwest Oncology Group (SWOG) trial S0003 (paclitaxel/carboplatin ± the hypoxic cytotoxin tirapazamine). Post-treatment plasma samples were available in 56 patients. **Results:** Baseline OPN plasma levels correlated significantly with patient overall survival (OS). High interpatient variability was observed, with levels ranging from undetectable to 2560 ng/ml, (median: 606.5 ng/ml). When dichotomized, median OS was 11 months for patients below median OPN levels & 7 months for those above ($p = 0.004$). Survival decreases with increasing OPN concentration. Furthermore, OPN levels correlated with response rate (RR) (median responders: 497; median non-responders: 698 ng/ml. Wilcoxon rank-sum $p = 0.03$). No association between baseline levels of either VEGF or PAI-1 with RR or OS was observed. However, plasma levels of both PAI-1 & VEGF were significantly inter-related & trended together ($p < 0.0001$), & both decreased significantly after treatment ($p = 0.0004$ & 0.04, respectively). Median decrease: OPN: 17%, PAI: 44%, VEGF: 42%. No significant differences were observed between study arms, suggesting that OPN is prognostic in NSCLC, but not predictive for response to tirapazamine. **Conclusions:** 1) There is a great need for development of tumor biomarkers which can be serially assessed pre- & post-therapy. 2) High OPN plasma levels were significantly associated with reduced RR & OS for patients on this trial. OPN is a strong candidate for inclusion in a panel of prognostic (& perhaps predictive) markers for NSCLC. Supported by the Hope Foundation & R01-CA107228.

7199 General Poster Session (Board #U8), Sun, 8:00 AM - 12:00 PM

The relationship between RASSF1A aberrant methylation and survival in small sized lung adenocarcinoma. *K. Miyajima, T. Ohira, J. Usuda, H. Saji, M. Tsuboi, T. Hirano, H. Kato, M. Suzuki, S. Toyooka, A. F. Gazdar; Tokyo Medical University, Tokyo, Japan; University of Texas Southwestern Medical Center, Dallas, TX*

Background: Aberrant methylation of CpG islands in promoter regions of tumor cells is one of the major mechanisms for silencing of tumor suppressor genes. The RAS association domain family 1A (RASSF1A) gene was isolated from the 3p21.3 region homozygously deleted in lung cancer cell lines, and it was shown to be inactivated by hypermethylation of the promoter region in lung cancers. In this study, we investigated the clinicopathological significances of RASSF1A methylation in the development and/or progression of small-sized (less than 2.0cm) lung adenocarcinoma. It is important to identify a marker for high-risk early stage patients who should benefit from new investigational adjuvant therapies. **Methods:** Surgically resected specimens from 77 cases of small-sized primary lung adenocarcinoma. We determined the frequency of aberrant promoter methylation of the RASSF1A genes in small-sized adenocarcinoma. Aberrant promoter methylation was examined using methylation-specific PCR (MSP). **Results:** Twenty-five of 77 (32.5%) tumors showed RASSF1A methylation. RASSF1A methylation was dominantly detected in smoker ($P < 0.03$). There was no significant correlation of RASSF1A methylation with gender, age, T stage, N stage and pathological stage. RASSF1A methylation correlated with adverse survival by univariate analysis ($P < 0.005$) as well as multivariate analysis ($P = 0.0062$; RR 4.251; 95% C.I., 1.507–11.993). Furthermore, RASSF1A promoter hypermethylation in resected stage I small-sized lung adenocarcinoma was associated with impaired patient survival ($P < 0.01$). **Conclusions:** Aberrant promoter methylation of the RASSF1A was present in 25 of 77 (32.5%) of small-sized lung adenocarcinoma by MSP assay. These results indicated that epigenetic inactivation of RASSF1A plays an important role in the progression of small-sized lung adenocarcinoma, and that RASSF1A hypermethylation appears to be a useful molecular marker for the prognosis of patients with small-sized and stage I lung adenocarcinoma. RASSF1A is a potential tumor suppressor gene that undergoes epigenetic inactivation in lung adenocarcinoma through hypermethylation of its promoter region. RASSF1A methylation was significantly related to unfavorable prognosis in small-sized lung adenocarcinoma.

F.V. Fossella・R. Komaki・J.B. Putnam,Jr. 編
加藤治文／池田徳彦／坪井正博 監訳

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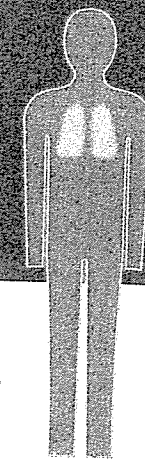

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