between IA and IB disease and that lymphatic invasion and vessel invasion have less impact on prognosis than VPI, suggesting that pleural lymphatic drainage is a more efficient pathway for metastasis [21]. The other reason is the rapidity with which a lung cancer in a subpleural location invades the pleura and disseminates cancer cells throughout the pleural cavity. Once exfoliated in the pleural cavity, subpleural lymphatics connecting with the pleural space could account for the lymphatic and then the subsequent systemic dissemination of the tumor cells [22]. In recurrence patterns of this study, VPI tumors developed a higher frequency of dissemination or malignant plural effusion than non-VPI in tumors ≤5 cm (≤3 cm, 9.2% vs. 0.9%, $p<0.0001$; 3.1–5 cm, 17.6% vs. 0.5%, $p<0.0001$; respectively; data not shown). VPI may be regarded as the first step toward minimal hematogenous tumor cell dissemination, regardless of regional lymph node metastasis.

Incidentally, a strong relationship between lymph node status and tumor diameter has been reported in NSCLC [23]. We therefore analyzed the relationships between tumor size or VPI and lymph node involvement (Fig. 3). In our present series, the percentages of lymph node metastasis in tumors ≤3 cm, 3.1–5 cm, and 5.1–7 cm were 13.3%, 30.4%, and 39.6%, respectively ($p<0.0001$) (data not shown). In tumors ≤3 cm, especially tumors ≤2 cm, VPI was significantly associated with an increased rate of lymph node metastasis ($p=0.0003$, and $p=0.015$, respectively). This significant association between VPI and lymph node metastasis was not found in tumors >3 cm in our study. There have been several reports showing little difference in survival when performing either mediastinal lymph node dissection or lymph node sampling in stage I NSCLC such as tumors ≤3 cm [24,25]. However, if there were preoperative or intraoperative VPI findings in small sized tumors, more extensive lymph node dissection may be needed rather than only lymph node sampling.

In any nodal status and node-negative patients of our subset analysis (Table 3a), the results indicate that VPI may not influence poor survival in patients with 5.1–7 cm tumors, although the limitation includes a small sized sample. The OS of patients with 3.1–5 cm/VPI (group D) was significantly worse than the OS of patients with 3.1–5 cm/non-VPI (group C) or ≤3 cm/VPI tumors (group B). No significant difference in survival outcomes between patients with 3.1–5 cm/VPI tumors and patients with T2b tumors (group E or F) were observed. On the other hand, although there was no statistically significant difference in survival outcome between patients with ≤3 cm/VPI tumors and patients with 3.1–5 cm/VPI ($p=0.07$), similar relationships were observed among these groups with N0 disease. This suggests that 3.1–5 cm/VPI tumors, which were classified as T2a, should be upstaged to T2b (Table 3b). Moreover, in the analysis of tumors ≤3 cm, although there was a significant difference in OS between non-VPI (group A) and VPI tumors (group B) in patients with any pN, there was no significant difference among these groups in the pN0 patients. This study does not statistically suggest that tumors ≤3 cm (T1a and T1b) with VPI are upgraded as T2a in the 7th edition. One possible reason is the statistically significant association between VPI and lymph node metastasis in tumors ≤3 cm. Thus, in our series of tumors without lymph node metastasis, there may be patients with occasionally good outcomes whose tumors had VPI but did not develop lymph node metastasis. In patients with tumors ≤3 cm, when analyzed according to pl0, pl1, and pl2, the percentages of pN0 patients were 89.1%, 76.9%, and 74.1%, respectively (pl0 vs. pl1, $p=0.002$; pl0 vs. pl2, $p=0.02$; pl1 vs. pl2, $p=0.83$) (data not shown). Pl2 was previously reported to be a more significant prognostic factor for worse OS than pl1 in node-negative NSCLC with VPI [26,27]. In tumors ≤3 cm without lymph node metastasis in the current study, the 5-year OS rates of pl0, pl1, and pl2 patients were 86.6%, 81.6%, and 63.4%, respectively (pl0 vs. pl1, $p=0.80$; pl0 vs. pl2, $p=0.008$; pl1 vs. pl2, $p=0.14$) (data not shown). Li et al. reported that there was no difference in survival between patients with or without VPI in stage I NCSLC, namely, tumors ≤3 cm [28]. Since there was also a significant difference in RFS between non-VPI and VPI tumors in patients with pN0, large-scale studies must be carried out to specifically clarify the impact of VPI on the OS of patients with tumors ≤3 cm.

The limitations of this study include its retrospective nature, which evaluated cases from 2000, a small sample size, and the fact that routine adjuvant chemotherapy for N1 or higher patients was started in 2004. These limitations potentially complicated the evaluation of the effects of VPI on outcome with respect to adjuvant chemotherapy.

In conclusion, despite the limitations mentioned above, we found that the presence of VPI was an independent prognostic factor for a poor prognosis in NSCLC patients. Moreover, 3.1–5 cm/VPI tumors should be upstaged to T2b tumors in the future edition of the TNM classification of the UICC staging system. In addition, the surgical strategy involving more extensive lymph node dissection for patients with ≤3 cm/VPI tumors, especially ≤2 cm/VPI, is warranted because of the high frequency of lymph node metastasis. As this may affect staging criteria, additional studies are needed to further clarify the underlying reasons why tumors with VPI have an unfavorable prognosis.

Financial support

This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (Grant no. 21791332) and the Ministry of Health, Labour and Welfare (Grant no. 22101601).

Conflict of interest statement

None declared.

Acknowledgements

We are indebted to Associate Professor E.F. Barroga and Professor J.P. Barron, Chairman of the Department of International Medical Communications of Tokyo Medical University for their editorial review of the English manuscript.

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Hindawi Publishing Corporation
International Journal of Proteomics
Volume 2012, Article ID 921901, 10 pages
doi:10.1155/2012/921901

Review Article

Cancer Phenotype Diagnosis and Drug Efficacy within Japanese Health Care

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Received 9 December 2011; Accepted 28 February 2012

Academic Editor: Tadashi Kondo

An overview on targeted personalized medicine is given describing the developments in Japan of lung cancer patients. These new targeted therapies with novel personalized medicine drugs require new implementations, in order to follow and monitor drug efficacy and outcome. Examples from IRESSA (Gefitinib) and TARCEVA (Erlotinib) treatments used in medication of lung cancer patients are presented. Lung cancer is one of the most common causes of cancer mortality in the world. The importance of both the quantification of disease progression, where diagnostic-related biomarkers are being implemented, in addition to the actual measurement of disease-specific mechanisms relating to pathway signalling activation of disease-progressive protein targets is summarised. An outline is also presented, describing changes and adaptations in Japan, meeting the rising costs and challenges. Today, urgent implementation of programs to address these needs has led to a rebuilding of the entire approach of medical evaluation and clinical care.

1. Health Care Costs and Impact

The rising cost in Japanese healthcare system, with an elderly population that is expected to reach 30% of the population by 2020, is a major challenge to the health care system (http://www.ipss.go.jp/syoushika/tohkei/Popular/Popular2011.asp?chap=0 in Japanese). This growth of elderly population in society is a trend that can be seen in other countries as well. The US, for instance, has an estimated growth of 16%, and Germany 23% by 2020. However, the Japanese situation is extreme in that 39.6% is predicted by 2050, which is far more than any other country in the world [1]. At the moment, Japan has most probably the largest future costs associated to the increasing elderly population. At the same time, the country has one of the lowest medical health care spends (in comparison to other developed countries) which is about 8.1%. This part of the budget is used on the national medical expense, based on the national GDP. These anticipated future costs and changes in society would be challenging the Japanese society for decades to come. To meet these alterations, it is envisioned that major changes will be implemented in emerging technologies and patient treatment procedures [2].

It is clear from a historical background that the future of biomedical sciences will be driven by the ability to adopt novel technologies, which will generate huge amounts of data outputs from clinical samples. One major consequence will be to utilize the new technology deliveries as the basis to understand the disease complexity and to develop new treatments. This is especially relevant to diseases such as lung cancer (LCa) and chronic obstructive pulmonary disease (COPD), the latter, a disease that is rapidly increasing and that presents itself in combination with LCa. These pulmonary diseases currently carry a huge mortality and

cost to the health care system. At the same time, these diseases have been shown to advance prognosis and reduced cost to healthcare system by early detection, prescription of personalized medicine, and evaluation of response to treatment. These diseases are known to be highly complex and multifactorial. It is not possible at this stage to assign a single molecule related to one disease or clinical complaint. On the contrary, there are hundreds (multiple signals), and there is a need of selecting from multiple signals. This is a highly demanding task, as this is hampered by the lack of tools and data for early diagnosis. In addition, modeling of disease progression and evaluation of treatment response is also something that the science community is still working on, and not a scientific tool that is available. LCa and COPD are both known to cluster in families and are more common in elderly population. Aggregation has been observed in families which would suggest a genetic or an environmental connection. Pathologically it has been observed that a lower lung function is seen in COPD patients, which would indicate a significant risk and a valuable predictor of incidents in lung cancer. We are experiencing that prevalence is increasing in patients with lung cancer, which is independent of age, sex, and smoking history. Consequently, there is a sixfold higher prevalence in lung cancer patients.

Currently, these disease areas are facing major challenges where major research resources are directed, such as the stratification of phenotypes along with an early indication of disease and diagnostics that can identify disease appearance and staging. With an optimal treatment, based on individual medical needs, is currently fundamental to the rebuilding of the entire medical and clinical system. This will be including the cases of lung tumors, their diagnosis, the surgical treatments, and/or chemotherapy of each individual subject.

In this respect, the concept of personalized medicine declared as a working proposition still is in its initial phase of developments and implementation worldwide. A major and unremitting effort is considered necessary to achieve these developments such as the requirement to establish the ranges of quantitative assays. These assays need to be able to separate healthy from diseased individuals in a variety of basic sciences such as genomics, proteomics, and metabolomics, as well as clinical sciences. Clinically, surveillance, diagnosis, and treatment should be in focus, which then is applied to match scenarios of individual disease with best practice individual treatment efficacy. Lately, a major focus of the introduction of targeted personalized medicine is marker associations to drug efficacy and safety. The targeted treatments are reducing costs for an aging Japanese population.

2. Cost and Benefits for the Japanese Patients

In many respects, Japan has been pioneering the optimal use of drugs for the Japanese population. This is especially highlighted by the successful use of personalized medicines for lung cancer treatments. The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are by now well-established treatment for advanced non-small-cell lung cancer (NSCLC). The advantage with these new generation of targeted drugs is that they in comparison to chemotherapy show that they are typically well tolerated and without cytotoxic side effects. The discovery of a novel class of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) was first made by AstraZeneca in 1994. EGFR is a target that is overexpressed in high levels on cancer cell surfaces in general, and particularly on non-small cell lung cancer (NSCLC) cells. Consequently, elevated levels of EGFR have been linked with progressed disease, cancer spread, and poor clinical prognosis. The tyrosine kinase enzyme(s) in the EGF receptor is inhibited by Gefitinib [3]. It results in a blocking effect of signaling that is linked to the functions of growth and spread of tumors. These types of tumors are predominantly effective with IRESSA treatments. The specific action of Gefitinib is linked to a high-affinity binding to the mutated EGFR tyrosine kinase domain with high specificity. A significant tumor shrinkage upon IRESSA treatment occurs in the majority of patients with EGFR mutation.

In this respect, both IRESSA (Gefitinib) and TARCEVA (Erlotinib) have been used by Japanese patients between 2002 and 2007, respectively, for treatment of advanced NSCLC [4]. AstraZeneca was the first pharma company that managed to get the targeted TKI, small molecule drugs to efficiently treat lung cancer patients. The chemical properties of Erlotinib and Gefitinib drug compounds are somewhat similar, while the chemical structures vary significantly, as shown in Figure 1.

These targeted small molecule drugs are used as oral monotherapy treatments. They have been proven to offer a superior quality of life compared with doublet chemotherapy (carboplatin/paclitaxel) as first-line treatment for EGFR mutation-positive advanced NSCLC. The personalized therapy approach was also recently proven to be applicable, not only to Japan, but also to Asians in general.

In Japan, rising costs have impacted on the framework of maintaining an efficient and effective healthcare system. Today, urgent implementation of programs to address this need has led to a rebuilding of the entire approach of medical evaluation and clinical care. Central to this realignment is the concept and practice of providing personalized medicine as an effective means for delivering effective care.

Currently, there are some reports on the costs involved in patient treatments utilizing patient diagnosis upon drug use that ultimately relate to the issue of responders versus nonresponders. This is an important consideration that gets more and more attention since the overall economy, that society needs to provide, is related to the costs of personalized medicines, which in most cases are significantly higher than many other traditional drugs. Davis et al. recently presented new developments and experiences that increasingly confirm the value of using personalized medicines [5]. From this paper, it has been concluded that protein- and genetic-biomarker diagnosis show increasingly high cost-effectivity, in providing the right medicine to the right patient at the first prescription. Recent medical savings was reported, where

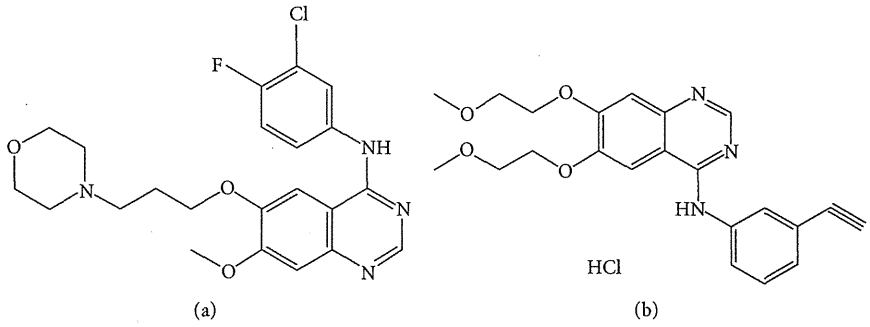


FIGURE 1: Chemical drug structure of Gefitinib, as a freebase (a) and Erlotinib as a (HCl) salt (b), respectively.

$40–80,000/year and patient is saved by the introduction of personalized medicine [5]. Safety considerations regarding possible side effects are also an added value, when patients show a negative drug test [6, 7].

As Japan currently is the world's largest market for tobacco products, consequently smoking related lung cancer's mortality has already been the highest among all cancers. These life conditions will have a severe impact on the quality of life of the smoke-induced and -related diseases in Japan. These effects are probably higher in Japan than in any other country, although the situation in China is alarming as well. In China, the combination of smoking, organic cooking, and the environmental factors are key drivers of lung cancer and COPD.

The tough challenge that the physician is faced with is to treat with an effective drug, and examples of these challenges have been reported and presented on at international congresses by our group over the years. The lung cancer phenotype is also of mandatory importance to correctly diagnose the cancer variant. Standard computer tomography (CT) imaging could efficiently be complemented by protein biomarker assays, such as multiplexing multiple reaction monitoring (MRM) assays [8, 9]. In addition, a multicenter study recently presented the solidity of the MRM technology platform [10].

Looking into the pipeline of coming drug products, currently in clinical phase trials, the expectation of new, targeted medicines, as well as antibody-based biopharmaceuticals, is expected to grow considerably. Biopharmaceutical medication such as in the treatment of NSCLC patients [11] revealed that Bevacizumab, a monoclonal antibody against vascular endothelial growth factor, has been shown to benefit patients with non-small-cell lung cancer resulting in a significant survival benefit [12].

The total market of targeted anticancer drugs in Japan, Europe, and United States has been continuously increasing which exceeded more than US$28 billion. Especially, regarding antibody drugs, their size in 2007 was around US$36 billion and estimated to increase up to US$67 billion in 2013. In Japan, about 10 antibody drugs were currently in market, which cover a total of 1 billion US$ (http://www.seedplanning.co.jp/press/2008/0610.html).

Personalized drugs have a natural Ying & Yang partnering effect within the treatment concept, which is the diagnostic marker that can assign an optimal drug treatment strategy that will benefit both the patient as well as the taxpayers.

The future optimal concept that healthcare institutions as well as politicians and industry are looking for is the following:

> *"The Right Medicine to The Right Patient at the Right Time Point."*

3. Impact to Society of Personalized Medicines

Genetic alterations of EGFR (exons 18, 19, 20, and 21) are important for predicting the efficacy of personalized medicine such as IRESSA in patients with lung cancer [13, 14].

The observation of EGFR somatic mutations in Japanese patients was made at a time point during the phase III study with IRESSA in North America [15]. The phase III study was not able to show a statistical significance of the drug with the criteria that were set at the time. However, previously, in the phase II study, the study outcome was highly successful with the dose and conditions given at the time, why AstraZeneca was urged to start production and distribution to patients before the phase III study was finalized. This was also the action that AstraZeneca took at the time. Nonetheless, at this stage, the subset of the Asian part of the patient cohort did prove a significant outcome upon IRESSA treatment, why the mode-of-drug action already at this time point was suspected to be related to EGFR mutation. The test to analyze the EGFR mutation status was developed that could identify one phenotype: mutated versus nonmutated [13]. These findings were associated with a long path of mechanistic development works. The resulting data from these studies and additional followup investigations resulted in a standard procedure using the EGFR-mutation assay, which could detect mutations in exon18 (G719A/C/S), exon19 (E746-A750 deletion, L747-P753 deletion insertion S), exon20 (S768I), and exon21 (L858I, L861Q) more than 1% mutation rate in the clinic today. In Japan, a positive indication will result in personalized medicine prescriptions [13, 16].

In protein expression research today, we discover, develop, and validate proteomics findings for new prospect applications in a clinical setting. As there is increasing funding globally available for research programs that can

improve the diagnosis and stratification of patients, as well as biomarkers for both safety assessments and efficacy, an increasing number of studies and study data that illustrate these developments are being published, as outlined in Figure 2.

Gefitinib (IRESSA, ZD1839) was developed as a specific inhibitor of the EGFR tyrosine kinase. Gefitinib targets the EGFR for therapeutic drug intervention within lung cancer. Early reports appeared on the experience of lung cancer patients with EGFR mutations. These were among the first clinical data that provided correlation with clinical response to Gefitinib therapy [16]. Personalized medicines have an improved efficacy over chemotherapy and radiation treatments. While targeted drugs can reach 70–75% efficacy, the combined effects of LCa chemotherapy and radiation will reach about 35%. With respect to the well-being of patients, the targeted drugs are not only more efficient but also provide an improvement in quality of life.

Recently a clinical study in four Asian countries was conducted: the IRESSA Pan-Asia Study, the "IPASS" study, in which 1,217 NSCLC patients were enrolled [17]. The IPASS study was an open label, randomised, parallel-group study that assessed the efficacy, safety, and tolerability of IRESSA versus doublet chemotherapy (carboplatin/paclitaxel) as first-line treatment in a clinically selected population of patients from Asia.

The adenocarcinoma cohort of the study with Japanese patients was enrolled for efficacy biomarker discovery. The ultimate objective is to identify responders to Gefitinib treatment to nonresponders. The final outcome for diagnostic prediction is still under investigation.

4. Clinical Biomarkers

There is a lack of protein biomarker and imaging diagnostics today within lung disorders, such as LCa and COPD. These new clinical tools are expected to be used as early indicator of disease, or as personalized indicator assays for targeted and stratified disease phenotype drug treatments in the near future. There is also a poor understanding of the mode of drug action mechanisms, by commonly used therapies, which is also true for new drugs introduced to the market. The actual targeted cells and proteins within disease and the actual drug interactions are by no means understood for most medicines used in today's therapies. These drug characteristics are needed for both efficacy and safety improvements and also requested by regulatory authorities, like FDA, MLHW, and EMEA.

Using a multiple biomarker approach such as proteomics (the simultaneous study of large parts of the human proteome to give a global view of differential expression of proteins in blood or tissue), rather than simply a conventional single biomarker, potentially increases predictive power both through increased robustness deriving from multiple measurements and the opportunity to combine information from multiple biological processes. To support high-quality generation of such information, we combined in a novel way several key study components: robust study design,

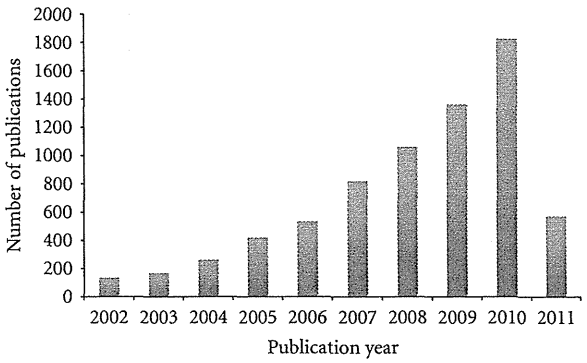


FIGURE 2: Publication frequency during the past 10 years using the keyword "clinical biomarker" in search of ISI Web of Knowledge on April 29, 2011.

well-defined phenotypic definitions, careful sample collection procedures, stable advanced liquid-chromatography (LC-) tandem mass spectrometry, MS/MS-based peptide separation and detection methods, and statistical analysis incorporating proteomic and clinical information. We developed stringent methods for database protein annotation of detected peptide peaks and biological interpretation using literature mining software, plus extensive quality control and validation in lung cancer studies. The first presentation on IRESSA biomarkers was presented at the European Cancer meeting (ECCO, Paris, France) in 2007 (http://www.ncbi.nlm.nih.gov/pubmed/16381621).

It is also expected that clinical chemistry diagnosis with biomarkers will become highly valuable as a screening platform with speedy and cheap diagnosis assays in relation to imaging, where, for instance, a typical pricing for CT or magnetic resonance imaging (MRI) in Japan is in the order of $10,000.

Mass-spectrometry-based proteomic approaches are based on digital molecular recognition (*m/z, mass over charge*) and sequencing in high resolution, and so those investigations of lung cancer have thrown light on understanding complexity of patient disease status, and from the clinicians' point of view, we believe that a utilization of useful biomarkers based on their quantitative molecular expressions can stratify patients and improve their therapeutic strategies for better clinical outcomes, and that such approaches would diminish unnecessary multiple treatments, which shall contribute to reduction of medical healthcare costs.

As the first country in the world, Japan has declared a pricing strategy that includes demands for biomarker diagnostics that should be used in combination with new drugs introduced into the market [18, 19]. Thereby, a targeted treatment with patient stratification will be achieved, with phenotype selection for responders to drug treatment [20].

In this declaration, pharmacogenomic and proteomic technologies are promoted in the discovery and development of drug-related biomarkers by the drug pricing committee within the MHLW (http://www.mhlw.go.jp/shingi/2009/07/dl/s0715-9a.239.pdf).

The overall aim of this declaration is to reach a Japanese pricing strategy that will be used in order to promote safe and efficient approved drugs for the treatment of Japanese patients.

4.1. Drug Safety Biomarker Studies. Stratification of patients who will benefit from Gefitinib treatment or even suffer side effects is major medical concern. A large Gefitinib postmarketing surveillance study in Japan (3,322 patients) reported 5.8% in rate of intestinal-lung-disease-(ILD-) type events. To reveal risk factors of ILD occurrence, a large-scale plasma proteomic study has been conducted in a cohort of NSCLC patients treated with Gefitinib [18, 19].

Reports have been coming from Japanese patients with non-small-cell lung cancer, treated with Gefitinib, that events of ILD appear some weeks after first administration of drug. ILD is a highly heterogeneous pathophysiology state that is not easily diagnosed.

ILD will affect the lung parenchyma and/or the alveolar region [21–23]. It was also presented by Kudoh's group that a high prevalence of drug-induced pneumonia in Japan has been identified in several studies [24–27]. Specific Gefitinib-related ILD occurrence has also been reported in a number of studies, where the occurrence frequency and predictive factors have been investigated [24–27].

A number of reports have been made over the last 4 years, where Gefitinib treated patients developing ILD were compared to a control group where the patients did not develop ILD [18]. In 2009, preliminary data were presented on safety biomarkers at the European Proteomics Association Congress (Stockholm, Sweden) from Japanese lung cancer patients undergoing IRESSA treatment. (http://www.eupa.org/EuPA2009/proceedings/6.Posters/2.%20Biomarker%20Proteomics%20and%20Applications%20%2874%29/P2-43.pdf).

The biomarker candidates were presented for the first time at the 26th ISPE Congress (International Conference on Pharmacoepidemiology and Therapeutic Risk Management) in 2010 in Brighton, UK (http://www.pharmacoepi.org/meetings/26thconf/26th_ICPE_Final_Program.pdf).

Recent followup patient analysis with plasma samples from the respective patient groups (CASES and CONTROLS of ILD) was analyzed by shotgun sequencing, utilizing the LC-MS proteomics platform [28, 29]. Typically, tens of thousands of mass signals generated from expressed protein sequences were detected, from where the differential expression analysis was performed. In parts of these studies, there was like ~7 million sequence data generated, with MS/MS fragmentations [30]. This is probably the largest clinical protein biomarker discovery study ever performed in the industry where personalized medicine treatments were made. The resulting outcome, in terms of protein sequence output and the clinical data within the IRESSA repository database, must also be one of the largest ever undertaken [30]. The future targeted drug treatments inking efficacy with safety was pioneered in Japan, and the improved quality of life for patients in addition to the cost benefit society will gain is illustrated in Figure 3.

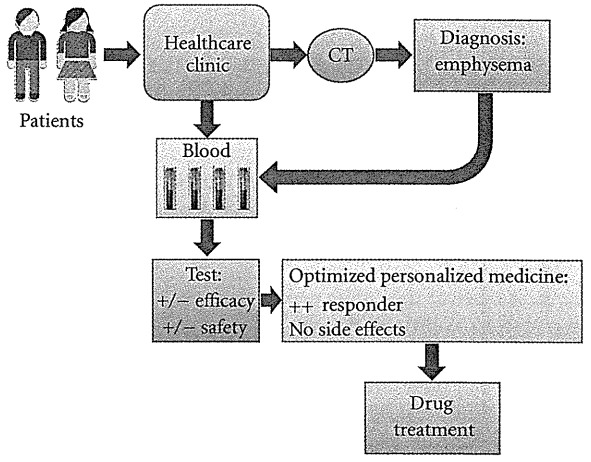


FIGURE 3: Diagnostic testing linked to targeted personalized drug treatment of lung cancer patients.

4.2. Specific Lung Cancer Phenotype Markers. Proteomic investigations of lung cancer use clinical materials such as frozen and formalin-fixed paraffin-embedded (FFPE) tissue specimens. Laser microdissecting the cancer cells from the pulmonary patient tissues is an efficient way to make: (i) drug-specific target discovery and (ii) biomarker discovery studies. It allows for expert pathological identification of the tumor cells in the tissue compartments, which is made by an electronic image analysis report. The areas within the tissues are marked by the pathologist. Next, the operator can use the marked image in order to make the laser microdissection and tumor cell isolation. This strategy allows for expert utility, where the experimental operators are distantly separated geographically.

Biomarker diagnosis studies were undertaken on large cell neuroendocrine carcinoma (LCNEC) patients, applying laser-microdissected tumor cell isolates [31]. LCNEC is a rather new lung cancer phenotype and has been categorized into one of the subtypes of large-cell carcinoma (LCC) [32]. The LCNEC phenotype, thus, was demonstrated to develop high-grade neuroendocrine tumors. Classifying the LCNEC phenotype is highly challenging, and as of now, there is no definite treatment for LCNEC patients. As LCNEC has not been found to be highly amenable to chemotherapy, as compared to small-cell lung carcinoma (SCLC), a diagnostic test is needed in order to separate the LCNEC patients from LC and SCLC. An additional complication is also that currently there is no specific marker for LCNEC that accurately identifies the disease evolvement, which allows for a targeted treatment strategy.

FFPE tissues have been archived in hospitals worldwide, together with detailed clinical records, for example, disease history, clinical examination results, drug response, and adverse reaction of individual patients. Recently, emerging technology for FFPE tissue proteomics has made it possible to study protein expression using FFPE tissue specimens, providing a great opportunity for biomarker discovery using clinically archived FFPE tissues accompanied by both definitive diagnoses and known clinical outcomes

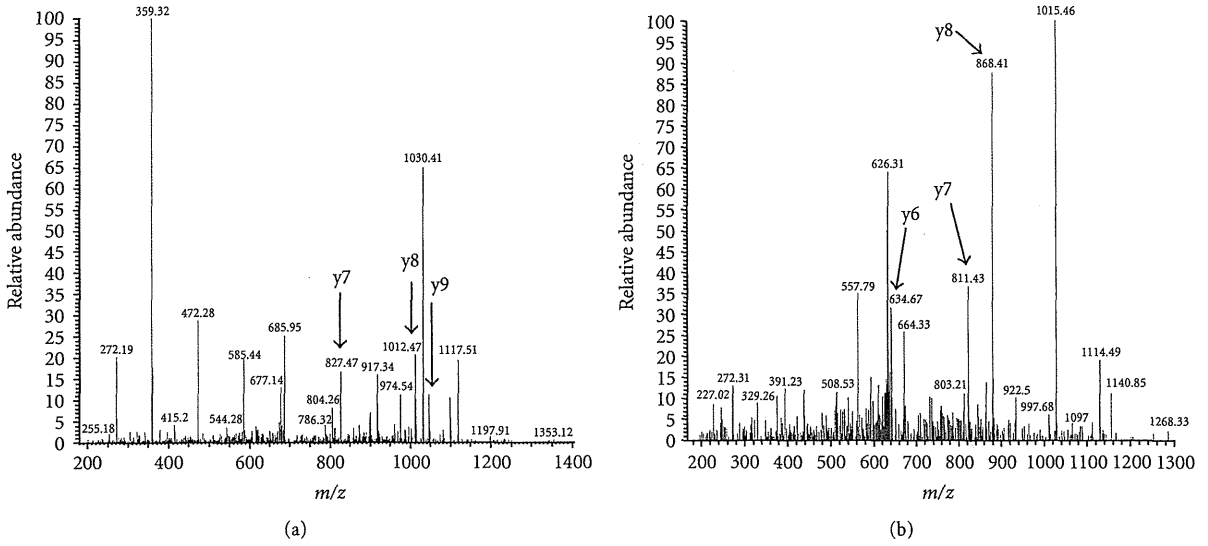


FIGURE 4: Mass spectra on the sequence identity of (a) Stathmin and (b) major vault protein (MVP) that was found differentially regulated in the LCNEC patient tissues.

[18, 19, 28, 33–35]. Our proteomic studies utilizing the laser-microdissected FFPE tissues of LCNEC, LCC and SCLC, resulted in more than 100 significant protein biomarker candidates [28]. Lately, MRM multiplex assay has become popular due to their generic applicability [36, 37]. The MS-based verification study of these candidates has been conducted by recent developments using MRM quantitative mass spectrometry as a novel methodology [29].

Following validation of biomarkers, MRM offers quantifications of proteins in complex biological matrices where key protein sequences are targeted within the assay [38]. In combination with appropriate stable isotope-labeled internal standards, the MRM assay technology provides absolute quantitation of the biomarkers. A great advantage is that a high number of proteins of interest can be monitored simultaneously within the MRM assay cycle.

MRM quantifications present high sensitivity and speed, which is a future requirement. High-throughput screening of clinical samples for candidate biomarkers within the clinical study area is the next frontier, where the MRM technology is developing further.

Our MS-based quantitative studies of those candidates have verified 44 promising biomarker candidates, using MRM [28]. Figure 4 shows the corresponding MS/MS spectra of (a) Stathmin and (b) major vault protein (MVP) from the corresponding MRM quantitation readouts. We discovered that Stathmin is highly expressed in neuroendocrine lung tumors (within the SCLC and LCNEC patient groups), and that MVP would be significant to LCC [18, 19]. It is expected that their subset of candidates would be useful for an improved differential diagnosis of LCNEC patients. These studies are undertaken by our research team as well as others throughout almost a decade. The studies undertaken in Japan are all performed in a clinical environment with close collaboration to the expertise of hospitals. The surgeons have provided the bed-side resected tissue samples for our studies, along with clinicians, that have been giving their experienced guiding for biofluid sampling, CT-imaging, and clinical demography data. In addition, we have been collaborating closely with pathologists who have performed the diagnosis of patients and been instrumental in our own proteomic studies, when comparing classical histology with proteomics-generated biomarker diagnosis predictions.

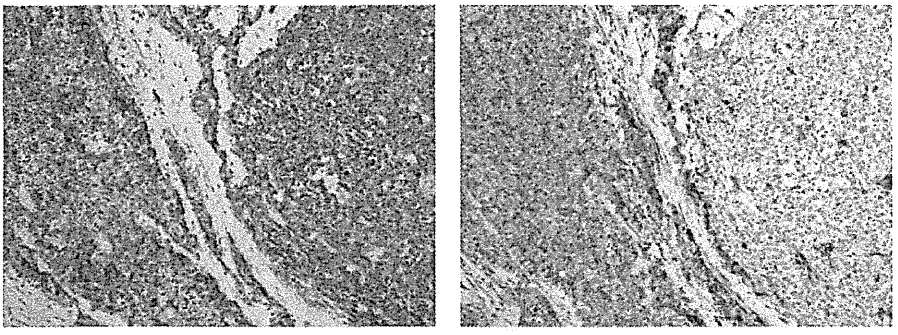
In view of this, the recently approved Neuroendocrine Lung Cancer group, which in terms of diagnosis is a highly challenging lung cancer phenotype to verify, will have novel biomarker candidates, that has the opportunity to exchange immunohistochemical identity of patients with protein sequencing assay technology.

Histology still remains the standard for pathology staging, used as a golden standard for protein biomarker diagnosis and proteomics [39], why the validation of the discovered biomarkers was run on additional LCNEC patients. The corresponding immunohistochemical identities were confirmed in these histology studies, shown in Figure 5.

5. Drug Localization in Tissue Compartments by Maldi Drug Imaging

The basic understanding of the disease developments and the pathophysiological landmarks that these changes leave is the starting point for an understanding of the molecular fingerprint that these disease stages establish. *In vivo* disease models developed for a given disease state, or rather a mimic of a patient group biology occurrence, are currently a rapidly growing research area. The translation from *in vivo* animal disease models and the predictive values in a human clinical study setting is a critical phase of drug development, establishing the patient dose levels.

Drug localization after administration is highly valuable clinical information, where both target specificity and



(a) (b)

FIGURE 5: Immunohistochemical staining of LCNEC tissue for (a) Stathmin and (b) for major vault protein (MVP).

redundancy can be determined. An understanding of the pharmacokinetic as well as pharmacodynamic properties of a drug is of mandatory importance in drug development processes. Imaging technologies are gaining more power in drug characterization, utilizing positron emission tomography (PET), MRI, MS-CT, and MALDI imaging. In order to increase the basic knowledge about drug substances and their pharmacological effects, MALDI-imaging model-based developments have been introduced lately, which includes cell based as well as tissue-based validations of drug compounds [40]. The distribution of TARCEVA in tumor tissues isolated from a lung cancer patient is illustrated in Figure 6.

6. Conclusions

It is envisioned that the upcoming generation of personalized drugs for targeted and stratified patient treatment will break through in major disease areas such as lifestyle-related cancers, in particular lung cancers that have the highest mortality including a predisposing disorder chronic obstructive pulmonary disease. Other cancers such as, for instance; breast, colon, malignant melanoma, and brain are expected to have targeted treatments in the coming years. In addition, cardiovascular diseases, neurodegenerative diseases such as multiple sclerosis (MSc), Parkinson, and Alzheimer, obesity, and diabetes are additional disease areas where a multitude of pipeline drugs are expected to make it as products into the market, and the shelves of the local pharmacy, readily available for patients. Mass spectrometric technologies can provide the "phenotypic fingerprint" required for the concept of personalized medicine. Mass-spectrometry-driven target biomarker diagnoses in combination with high-resolution computed tomography can provide a critical pathway initiative facilitated by a fully integrated e-Health infrastructure system.

Expert Commentary

Targeted drugs, as the new generation of medicines, are expected to be used in combination with diagnostics, in order to increase efficient treatment of patients. It is also to be expected that the strong increase in biomarker developments will play a significant role in these strategic changes that will be of huge benefits to the health care as well as to the society and taxpayers. With technology developments associated to the personalized treatment, the clinical chemistry unit of future hospitals will also be making an increased number of analysis and assays in order to provide stronger diagnosis basis for the doctors at hospitals to make improved disease diagnosis of the patient.

We will also see a fast development of higher throughput diagnostic assays, where the breakthrough of the human genome and the human proteome will be used as the basis for these developments.

A Five-Year View

We do expect that during a five-year period we have reached a point where protein-based biopharmaceuticals or biologicals have taken a larger percentage of the drug market with a targeted drug approach. Improved clinical chemistry diagnosis with gene- and protein-sequence-based assays will also become state of the art in future medical health care that will result in a much higher number of overall measuring points. High-throughput multiplexed biomarker assay platforms will play an important clinical role as becoming a complement to traditional immunoassays for future use in clinical health care and targeted medicine. The introduction of new biomarkers of tumors, emphysema, and inflammatory reaction in the lung will assist in early identification of disease and in monitoring the effect of therapeutic agents on disease progression.

Key Issues

(i) Within lung cancer, there have been reports on early indication of somatic mutation appearances within the EGF receptor, where personalized drugs in Japan hve been proven highly efficient.

(ii) Personalized medicine is more cost-effective for society, with an overall benefit for the patients as well as for the tax payers.

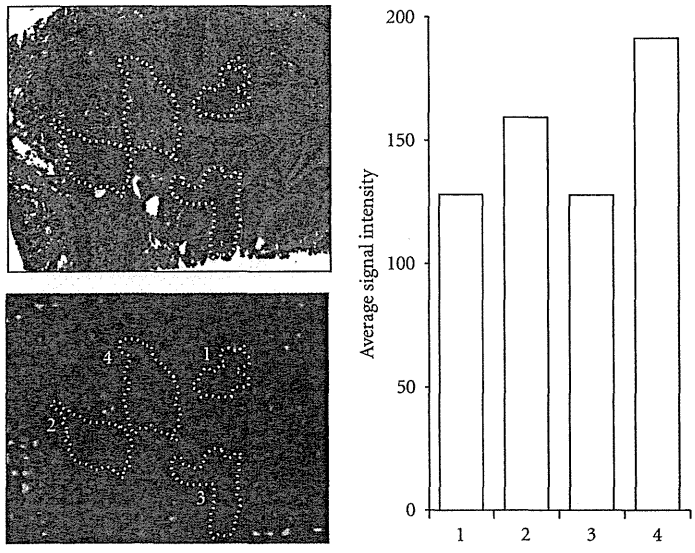


FIGURE 6: Enlarged region of a squamous cell lung tumor section with MALDI-MS read out of the Erlotinib fragment ion (m/z 336.19) and HE stained histological details. Typical areas of tumor cells are indicated with yellow-dashed lines.

(iii) Japan has declared a pricing strategy that includes request for new biomarker diagnostics that can be used for patient stratification.

(iv) We will also see a fast development of higher throughput diagnostic assays, where this breakthrough will become a new milestone in modern healthcare.

Abbreviations

2-DE:	Two-dimensional gel electrophoresis
COPD:	Chronic obstructive pulmonary disease
CT:	Computer tomography
EGFR:	Epidermal growth factor receptor
EMEA:	European Medicines Agency
FDA:	Food and Drug Administration
FFPE:	Formalin-fixed paraffin embedding
IHC:	Immuno-histochemistry
ILD:	Intestinal lung disease
LCa:	Lung cancer
LCC:	Large cell carcinoma
LC-MS:	Liquid chromatography-mass spectrometry
LCNEC:	Large cell neuroendocrine lung cancer
MALDI-MSI:	Matrix-assisted laser desorption/ionization mass spectrometric imaging
MHLW:	Ministry of Health, Labour and Welfare
MRI:	Magnetic resonance imaging
MRM:	Multiple reaction monitoring
MSc:	Multiple sclerosis
NSCLC:	Non-small cell lung cancer
PAC:	Post-operative adjuvant chemotherapy
PET:	Positron emission tomography
SCLC:	Small cell lung carcinoma
TKI:	Tyrosine kinase inhibitor
WHO:	World Health Organization.

Acknowledgments

The authors would like to thank Thermo Fisher Scientific for mass spectrometry support. The authors are grateful for funding support from the Swedish Research Council, Vinnova and Foundation for Strategic Research—the programme: Biomedical Engineering for Better Health—Grant no. 2006-7600 and Grant no. K2009-54X-20095-04-3, Inga-Britt Lundberg Foundation, Knut and Alice Wallenberg Foundation, and the Crafoord Foundation.

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World J Surg (2012) 36:2865–2871
DOI 10.1007/s00268-012-1743-5

World Journal of Surgery

Retrospective Analysis of Nodal Spread Patterns According to Tumor Location in Pathological N2 Non-small Cell Lung Cancer

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Published online: 5 September 2012

Abstract
Background The purpose of the present study was to determine the nodal spread patterns of pN2 non-small cell lung cancer (NSCLC) according to tumor location, and to attempt to evaluate the possible indications of selective lymph node dissection (SLND).
Methods We retrospectively analyzed nodal spread patterns in 207 patients with NSCLC of less than 5 cm with N2 involvement.
Results The tumor location was right upper lobe (RUL) in 79, middle lobe in 12, right lower lobe (RLL) in 40, left upper division (LUD) in 41, lingular division in 11, and left lower lobe (LLL) in 24. Both RUL and LUD tumors showed a higher incidence of upper mediastinal (UM) involvement (96 and 100 %, respectively) and a lower incidence of subcarinal involvement (15 and 10 %, respectively) than lower lobe tumors (UM; RLL 60 %, LLL 42 %; subcarinal: RLL 60 %, LLL 46 %, respectively). Among the patients with 24 right UM-positive RLL and 10 left UM-positive LLL tumors, 2 showed negative hilar, subcarinal, and lower mediastinal involvement, and cT1, suggesting that UM dissection may be unnecessary in lower lobe tumors with no metastasis to hilar, subcarinal, and lower mediastinal nodes on frozen sections according to the preoperative T status. Among the patients with 12 subcarinal-positive RUL and 4 subcarinal-positive LUD tumors, one showed negative hilar or UM involvement, suggesting that subcarinal dissection may be unnecessary in RUL or LUD tumors with no metastasis to hilar and UM nodes on frozen sections.
Conclusions The present study appears to provide one of the supportive results regarding the treatment strategies for tumor location-specific SLND.

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Introduction

Lobectomy with systematic mediastinal lymph node dissection (LND) has been considered the standard of care for resectable non-small cell lung cancer (NSCLC). Lymph node dissection was first reported by Cahan in 1960 [1] and is known to enhance staging accuracy by increasing lymph node harvesting and improving the identification of occult N2 disease. In contrast, other investigators claim that LND can potentially increased postoperative morbidity or may require longer operative time [2–5]. Some randomized controlled trials addressing the survival benefit of LND and mediastinal lymph node sampling showed no difference in survival outcome between patients undergoing LND and those undergoing lymph node sampling [3, 6, 7]. Whether or not patient outcome is improved by LND remains controversial.

At present, early lung cancers are more frequently encountered because of the widespread use of high-resolution computed tomography (CT) in routine practice and cancer screening [8, 9]. Therefore, the extent of LND should be tailored to each patient. Selective lymph node dissection (SLND) based on the tumor location-specific lymphatic pathway should be undertaken especially for patients with no apparent lymph node metastasis or with impaired pulmonary function, or for elderly patients. In the present study, we retrospectively reviewed the prevalence of lymph node involvement in each mediastinal region in

patients with N2 NSCLC according to the location of the primary tumor, and we attempted to evaluate the possible indications for SLND.

Patients and methods

Patients

From January 1990 to December 2007, a total of 2,195 patients underwent radical surgical resection of at least a lobectomy and systematic LND for NSCLC at our hospital. Of these 2,195 patients, we retrospectively analyzed lymph node spread patterns and outcome in 207 patients with NSCLC of less than 5 cm with N2 involvement. We excluded patients who had received preoperative treatment, including chemotherapy or chemoradiotherapy, those who had undergone only biopsy and SLND, and those who had low-grade malignant tumors. We also excluded patients with tumors spreading across lobar fissures and invading multiple lobes.

Preoperative evaluation included physical examination, chest radiography, computed tomography (CT) of the chest and abdomen, magnetic resonance imaging of the brain, bone scintigraphy, and blood examination. We determined that a large lymph node over 10 mm in the shortest axis was positive for metastasis on CT scans. Positron-emission tomography (PET) scan (recently integrated PET-CT scan) was not routinely used for staging resectable tumors during the study period. In recent years, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) was sometimes performed for the patients having suspected multiple N2 lymph node metastases, but it was not routinely used. Similarly, mediastinoscopic biopsy was not routinely performed. Patients with N2 lymph node positively diagnosed by EBUS-TBNA or mediastinoscopic biopsy were excluded from the group of operative indication candidates.

The stage of disease was determined according to the 2009 7th Edition of the TNM Classification for Lung and Pleural Tumors [10]. The institutional review board of our institution approved the data collection and analyses and waived the need to obtain written informed consent from each patient.

Operation

During thoracotomy, lymph nodes in the ipsilateral thoracic cavity were completely resected. Systematic nodal dissection, including the superior to inferior mediastinum, was then performed after pulmonary resection. In cases of left thoracotomy, upper mediastinal dissection indicated aortic and tracheobronchial node dissection. If intraoperative findings showed that hilar or mediastinal lymph nodes were highly suspicious for metastatic disease, the resected lymph node specimens were immediately examined pathologically in frozen sections. Whether or not the presence or absence of lymph node metastasis was judged by intraoperative diagnosis, systematic LND was performed in the present study patients. Mediastinal metastases were considered to be skip metastases if any of the N2 nodes, but not the N1 nodes, were involved.

Mediastinal lymph node stations were grouped into the "zones" proposed by the International Association for the Study of Lung Cancer (IASLC) lung cancer staging project [11]. We also reviewed the correlation between nodal zone spread pattern and tumor location. We classified lymph node stations into the following six zones: the right upper (RU) and left upper (LU) zones, each including #2R, #3a, #3p, and #4R nodes; the subcarinal (SC) zone, including #7 nodes; the right lower (RL) and left lower (LL) zones, each including #8 and #9 nodes; and the aortic-pulmonary (AP) zone, including #5, and #6 nodes.

Statistical analysis

Overall survival time was measured from the date of surgery to the date of death from any cause or the date on which the patient was last known to be alive. Survival curves were plotted according to the Kaplan–Meier method and compared with the log-rank test. Two-category comparison was performed by the Pearson χ^2 test and Fisher's exact test for quantitative data. All tests were two-sided, and p values of <0.05 were considered to indicate statistically significant differences. We used StatView 5.0 (SAS Institute Inc., Cary, NC) for the statistical analysis.

Results

Patient characteristics are summarized in Table 1. Of the 207 patients with NSCLC of less than 5 cm with N2 involvement, 55 (27 %) had skip metastasis, and 97 (47 %) had both hilar and the remaining 55 patients had metastatic segmental lymph nodes or subsegmenta lymph nodes with mediastinal lymph nodes metastasis. In addition, 74 (36 %) were diagnosed with cN2 disease by the chest CT. Lymph node spread patterns according to primary tumor location are presented in Fig. 1. The most common site of involvement for tumors of the right upper lobe (RUL; $n = 79$) was the RU zone ($n = 76$; Fig. 1a). Right upper lobe tumors showed a significantly higher incidence of RU zone metastasis than right lower lobe (RLL) tumors (96 vs. 60 %, $p < 0.001$: Fig. 1a, b). In contrast, when RU zone metastasis was present, RLL tumors showed a significantly higher incidence of simultaneous metastasis to the SC or RL zone

Table 1 Patient characteristics ($n = 207$)

	n	(%)
Overall	207	(100)
Sex		
Male	134	(65)
Female	73	(35)
Histologic type		
Adenocarcinoma	149	(72)
Squamous cell carcinoma	41	(20)
Others	17	(8)
Tumor size (cm)		
2.0	38	(18)
2.1–3.0	55	(27)
3.1–5.0	114	(55)
p-T status		
pT1	47	(23)
pT2	129	(62)
pT3	18	(9)
pT4	13	(6)
Hilar lymph node metastasis		
Present	97	(47)
Absent	110	(53)
Skip metastasis		
Present	55	(27)
Absent	152	(73)
Tumor location		
Right upper lobe	79	(38)
Right middle lobe	12	(6)
Right lower lobe	40	(19)
Left upper division	41	(20)
Left lingular division	11	(5)
Left lower lobe	24	(12)
Procedure		
Pneumonectomy	15	(7)
Bilobectomy	19	(9)
Lobectomy	173	(84)

than RUL tumors (28 vs. 11 %, $p = 0.026$: Fig. 1a, b). The incidence of skip metastasis to only the RU zone was statistically lower among patients with RLL tumors than among those with RUL tumors (8 vs. 30 %, $p = 0.005$: Fig. 1a, b). Right upper lobe tumors showed a significantly lower incidence of SC zone metastasis than RLL tumors (15 vs. 60 %, $p < 0.001$: Fig. 1c, d). Most RUL tumors with SC zone metastasis showed simultaneous metastasis to the RU zone or hilar lymph nodes, and only one patient showed skip metastasis to the SC zone (Fig. 1c).

The most common site of involvement for tumors of the left upper division (LUD) ($n = 41$) was the AP or LU zone ($n = 41$; 100 %: Fig. 1e). Left upper division tumors showed a significantly higher incidence of AP or LU zone metastasis than left lower lobe (LLL) tumors (100 vs. 42 %, $p < 0.001$: Fig. 1e, g). In contrast, when AP or LU zone metastasis was present, LLL tumors showed a higher incidence of simultaneous metastasis to the SC or LL zone than LUD tumors, but the difference was not significant (29 vs. 12 %, $p = 0.089$: Fig. 1e, g). The incidence of skip metastasis to only the AP or LU zone was 45 % in left lingular division tumors, 20 % in LUD tumors, and 0 % in LLL tumors, but the difference was not significant (Fig. 1e–g). Left upper division tumors showed a significantly lower incidence of SC zone metastasis than LLL tumors (10 vs. 46 %, $p < 0.001$: Fig. 1h, j). All LUD tumors with SC zone metastasis showed simultaneous metastasis to the AP or LU zone, but no patient showed skip metastasis to the SC zone (Fig. 1h).

Patients were further categorized as those with tumors of the lower lobes ($n = 64$; 40 of right and 24 of left) and those with RUL or LUD tumors ($n = 120$; 79 of RUL and 41 of LUD). The prognosis of patients with lower lobe tumors and RUL or LUD tumors was analyzed. The 5-year overall survival (OS) rates of patients with tumors of the lower lobes with upper mediastinal metastasis ($n = 34$, 22 %) were poorer than, but not significantly different from, those of the patients without upper mediastinal metastasis ($n = 30$, 34 %) ($p = 0.371$; Fig. 2). The 5-year OS rates of patients with RUL or LUD tumors with SC zone metastasis ($n = 16$, 14 %) were poorer than, but not significantly different from, those of the patients without SC zone metastasis ($n = 104$, 40 %) ($p = 0.073$; Fig. 3).

The combined treatment strategies for tumor location-specific SLND in N2 NSCLC patients according to clinical T status are summarized in Table 2. Among 24 patients with upper mediastinal metastasis from RLL tumors, nine showed no evidence of hilar, SC zone, and lower mediastinal metastasis. Of these nine patients, only one had clinical T1. Similarly, among ten patients with upper mediastinal metastasis from LLL tumors, only one showed no evidence of hilar, SC zone, and lower mediastinal metastasis, and clinical T1 status. Upper mediastinal dissection may be unnecessary in lower lobe tumors with negative hilar, SC and lower mediastinal nodes on frozen sections if the preoperative T status is T1 (Table 3). In contrast, among 12 patients with SC zone metastasis from RUL tumors, one showed no evidence of hilar or RU zone metastasis, and that tumor was classified as clinical T2. Among four patients with SC zone metastasis from LUD tumors, none showed evidence of hilar, upper mediastinal metastasis. This finding supports the hypothesis that SC dissection may be unnecessary in RUL and LUD tumors with no metastasis to hilar and upper mediastinal nodes on frozen sections, regardless of the clinical T status. Figure 4 shows diagrams of the main pathways of lymphatic spread of tumors according to tumor location.

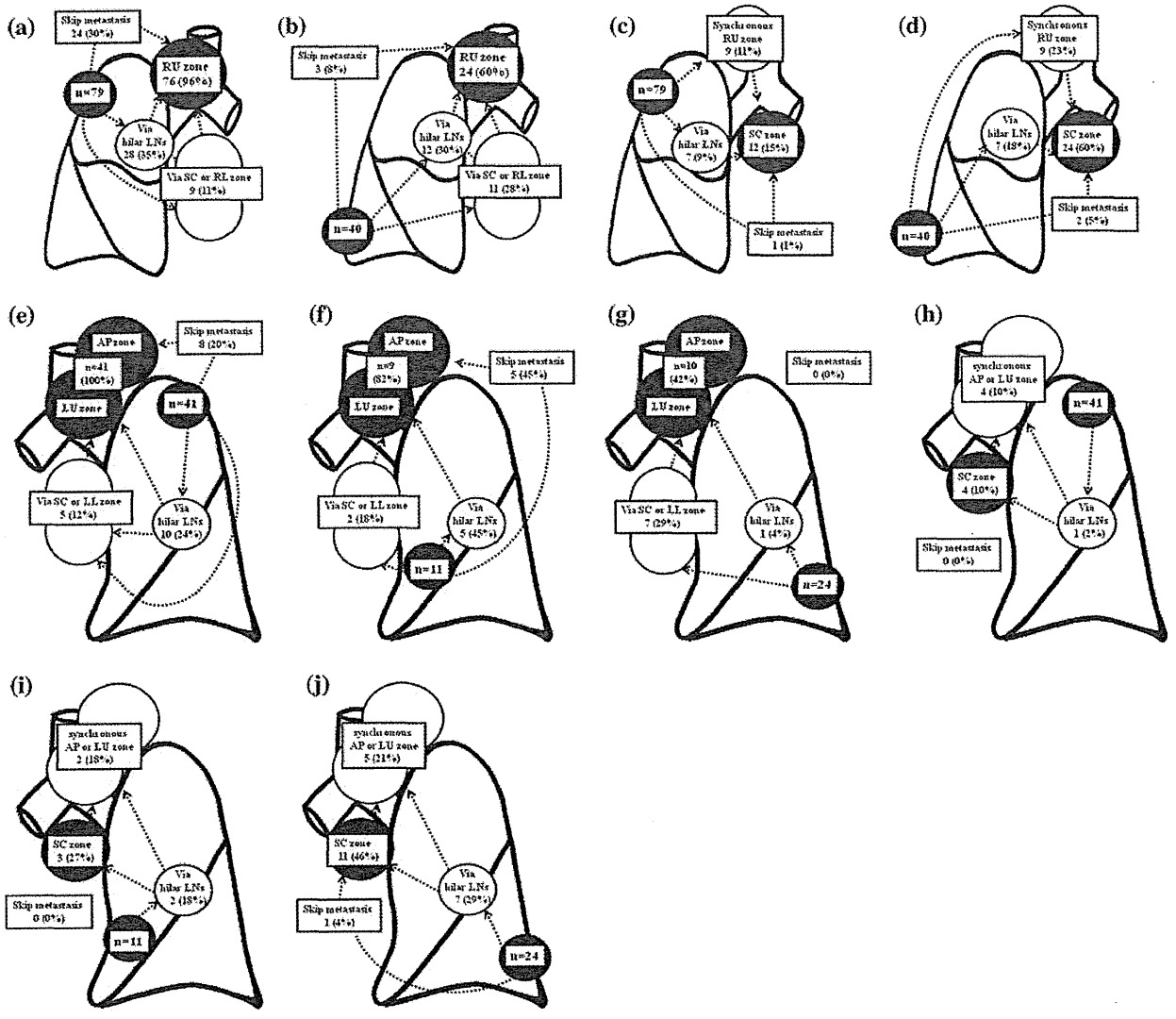

Fig. 1 Lymph node spread patterns according to the primary tumor location: **a** tumors of the right upper lobe (RUL) and right upper mediastinal metastasis. **b** Tumors of the right lower lobe (RLL) and right upper mediastinal metastasis. **c** Tumors of RUL and subcarinal metastasis. **d** Tumors of RLL and subcarinal metastasis. **e** Tumors of the left upper division (LUD) and left upper mediastinal metastasis. **f** Tumors of the left lingular division (LLD) and left upper mediastinal metastasis. **g** Tumors of the left lower lobe (LLL) and left upper mediastinal metastasis. **h** Tumors of LUD and subcarinal metastasis. **i** Tumors of LLD and subcarinal metastasis. **j** Tumors of LLL and subcarinal metastasis

Discussion

We set out to gain insight into the prevalence of lymph node metastasis in each mediastinal region in patients with pN2 NSCLC. The lymphatic pathways by which metastases from primary tumors in various segments and lobes spread toward the hilar and mediastinal lymph nodes have been investigated for over 50 years [12]. Studies of the patterns of location-specific lymphatic pathways of the lung have led to a better understanding of the importance of lymph node staging in the management of lung cancers. Although systematic LND consistently yields precise staging information, it may contradict the concept of the optimal extent of lymph node dissection based on the location of the tumor. Some authors have postulated that the dissection of lymph nodes without cancer cells causes higher morbidity and mortality because it extends the operative procedure [2, 6]. Moreover, the significance of LND regarding long-term outcome is still controversial. We therefore retrospectively reviewed the prevalence of mediastinal lymph node involvement in 207 patients with NSCLC of less than 5 cm with N2 involvement based on the location of the primary tumor, and we set out to determine the possible indications of location-specific SLND.

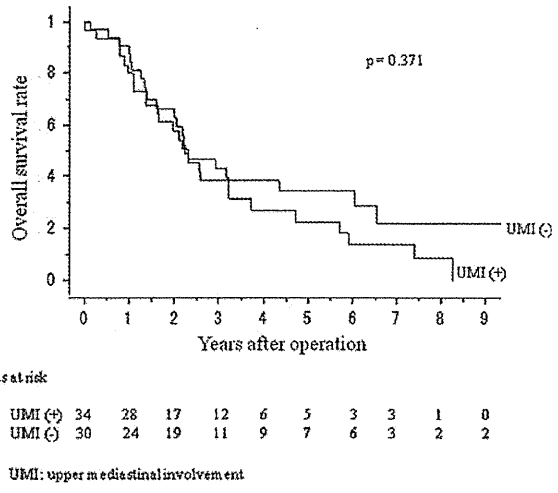

Fig. 2 Overall survival curves of lower lobe non-small cell lung cancer (NSCLC) pN2 patients, with or without upper mediastinal metastasis

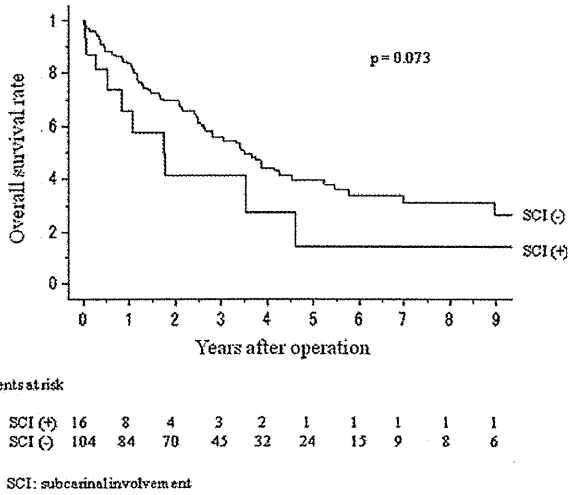

Fig. 3 Overall survival curves of right upper lobe or left upper division NSCLC pN2 patients, with or without subcarinal metastasis

Table 2 Strategy for tumor location-specific selective nodal dissection in N2 non-small cell lung cancer (NSCLC) patients: distribution of upper mediastinal involvement according to clinical T status

Tumor location					
	RUL *n* (%)	RLL	LUD	LLD	LLL
No. of patients with N2	79 (100)	40 (100)	41 (100)	11 (100)	24 (100)
No. of patients with UMI	76 (96)	24 (60)	41 (100)	9 (82)	10 (42)
Patients with UMI					
HI (–), SCI (–), LMI (–)	44 (56)	9 (23)	22 (54)	5 (45)	2 (8)
Clinical T1	14 (18)	1 (4)	5 (12)	2 (18)	1 (4)
Clinical T2–4	30 (38)	8 (21)	17 (41)	3 (27)	1 (4)

RUL right upper lobe, *RLL* right lower lobe, *LUD* left upper division, *LLD* left lingular division, *LLL* left lower lobe, *UMI* upper mediastinal involvement, *HI* hilar lymph node involvement, *SCI* subcarinal involvement, *LMI* lower mediastinal involvement

Table 3 Strategy for tumor location-specific selective nodal dissection in N2 NSCLC patients: distribution of subcarinal involvement according to clinical T status

Tumor location					
	RUL *n* (%)	RLL	LUD	LLD	LLL
No. of patients with N2	79 (100)	40 (100)	41 (100)	11 (100)	24 (100)
No. of patients with SCI	12 (15)	24 (60)	4 (10)	3 (27)	11 (46)
Patients with SCI					
HI (–), UMI (–)	1 (1)	3 (8)	0 (0)	0 (0)	3 (13)
Clinical T1	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)
Clinical T2–4	1 (1)	2 (5)	0 (0)	0 (0)	3 (13)

The IASLC staging project proposed the zone classification for future survival analyses [11]. Lee et al. [13] reported that grouping patients together according to zones provides accurate prognostic stratification for patients, and may resolve the ambiguity of the anatomical border, indicating applicability in the clinical setting. Therefore, we used the lymph node zone classification in this study.

Several retrospective studies have shown patterns of mediastinal lymph node metastases in relation to the location of the primary tumor [14–19]. Most of these studies have demonstrated that mediastinal lymph node metastases from RUL tumors occur predominantly in the RU area, but rarely in the SC area, whereas those from left upper lobe tumors occur most frequently in the AP or LU area, but those from tumors of the lower lobes rarely occur in the upper mediastinal area. In the present study, metastases to the SC zone from RUL or LUD tumors were significantly less frequent (15 and 12 %, respectively) than metastases to the SC zone from tumors of the lower lobes. The outcome of patients with RUL or LUD tumors with SC zone metastasis was poorer than, but not significantly different from, that of patients with RUL and LUD tumors without SC zone metastasis ($p = 0.073$). There was only 1 patient with only SC zone skip metastasis. Patients with upper lobe NSCLC involving SC nodes are reportedly rare [16, 18, 19], and they have poorer outcomes than those

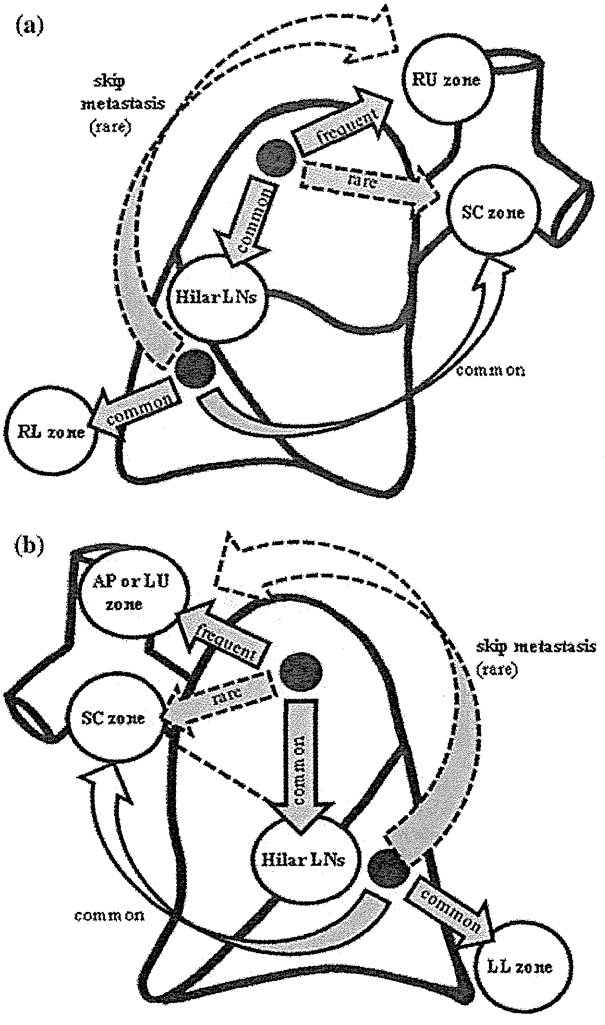

Fig. 4 Diagrams of the main pathways of lymphatic spread according to tumor location. **a** In right-side tumors, almost all RUL tumors metastasized to the RU zone directly or through the hilar lymph node. RUL tumors metastasized less frequently to the SC zone. *Right lower lobe* (RLL) tumors metastasized to various mediastinal lymph node zones, and skip metastasis to the RU zone was rare in RLL tumors. **b** In left-side tumors, all LUD tumors metastasized to the AP zone directly or through the hilar lymph node. *Upper lobe* tumors metastasized less frequently to the SC zone. *Left lower lobe* (LLL) tumors metastasized to various mediastinal lymph node zones, and skip metastasis to the AP zone was rare in LLL tumors

without SC node metastasis [19]. Based on these results, we also evaluated the possible indications of tumor location-specific SLND. Although we did not routinely perform frozen section diagnosis of sampled hilar lymph nodes, we conducted a frozen section examination intraoperatively if metastasis was suspected. There was only 1 patient with SC zone metastasis from RUL tumors who did not show any evidence of hilar and RU zone metastases, whereas no SC zone metastasis from any LUD tumors was observed when neither the hilar nor RU zone showed any evidence of metastasis. Resection of the SC zone in the case of RUL and LUD tumors may be unnecessary if neither upper mediastinal nor hilar lymph nodes show any evidence of metastasis on frozen sections, regardless of the clinical T status.

There were fewer patients with metastases to the upper mediastinal zone from tumors of the lower lobes than with metastases to the upper mediastinal zone from tumors of the upper lobes. The outcome of patients with tumors of the lower lobes with upper mediastinal metastasis was poorer than, but not significantly different from, that of patients with tumors of the lower lobes without upper mediastinal metastasis ($p = 0.371$). There was only one patient each with RU zone metastasis from a clinical T1 RLL tumor and AP zone metastasis from a clinical T1 LLL tumor, but neither showed any evidence of lymph node metastasis to the SC node, lower mediastinal zone, and hilum. Therefore resection of upper mediastinal zones in tumors of the lower lobes may be unnecessary even if the preoperative T status is T1, and if lymph node biopsies in the SC node, lower mediastinal zone, and hilum do not show any evidence of metastasis on frozen sections. However, former studies indicated that the superior and basal segment lung cancers in the lower lobe have different lymph node metastasis patterns [14]. Although there was no significant difference in the metastasis patterns of lower lobe tumors, this finding may be attributable to the small number of patients in the present study (data not shown). The strategy of lymph node dissection should be changed from extensive dissection to SLND, especially in early stage cancer or poor-risk patients, because SLND can reduce postoperative morbidity associated with such complications as bronchopleural fistula, chylothorax, or recurrent nerve palsy [2–5]. However, lung cancer can easily metastasize to the mediastinum, and therefore patient selection should be determined carefully. If patients are suspected of having advanced disease based on intraoperative findings, LND should be performed.

The present study has several limitations. It was a retrospective study, and possible bias may exist. First, we examined suspected hilar or mediastinal lymph nodes intraoperatively in frozen sections, but specific systemic sampling methodologies have been established and used in the past. Second, the number of patients in this study may be too small to draw any definitive conclusion. Third, current less-invasive staging modalities, including PET-CT or EBUS were infrequently used because of the inclusion of a large amount of data from old cases, collected at a time when these procedures were less well established. Thus we might have inadvertently performed some operations on undetected N3 disease.

In conclusion, we demonstrated the potential validity of refraining from resecting lymph nodes in the SC zone in

cases of RUL or LUD tumors, or those in the upper mediastinal zone in the case of tumors of the lower lobes. Considering the fact that NSCLC patients can benefit from SLND, a prospective study is essential to confirm the effect of tumor location-specific SLND on survival and optimal postoperative treatment.

Acknowledgments The authors are grateful to Mr. Roderick J. Turner, Assistant Professor Edward F. Barroga, and Professor J. Patrick Barron, Chairman of the Department of International Medical Communications of Tokyo Medical University, for their editorial review of the English manuscript.

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