表 I-4-3　ウシ血管内皮細胞の増殖，遊走および管腔形成に及ぼすMMP 阻害薬の効果[a)]

MMP 阻害薬	増殖活性	遊走活性[b)]	管腔形成[c)]
なし	100%	100%	100%
marimastat (10 μM)	100%	75%	20%
batimastat (10 μM)	100%	80%	40%
OPB-3206 (10 μM)	100%	80%	28%

a)：Shono ら[10)] 参照
b)：bFGF 存在下の遊走活性
c)：bFGF 存在下のコラーゲンゲル中の管腔様構造形成活性

害薬は，血管内皮細胞の増殖にはまったく影響を与えない濃度で著明に細胞遊走や管腔様構造の形成を阻害することが観察された(表 I-4-3)．このことは，MMP が血管内皮細胞遊走や血管構築に特異的に働いていることを示している．さらに MMP 阻害薬ががんの増大や転移，浸潤および血管新生を阻害することが明らかにされている．

3. BAY12-9566

marimastat の臨床試験では 381 人の治療不能とされる進行がん患者を対象とした第II相試験が行われ，がん抗原を指標とした応答率は 40～70%であり，非応答患者と比較して応答患者の延命率は有意に向上したことが報告された．しかし，単剤，また他の抗がん剤との併用で行われた世界的規模での第III相試験で，長期間服用では予想だにされなかった筋肉痛や関節痛などの副作用のため承認されなかった．他方，膵癌の臨床試験において，MMP 阻害薬である BAY 12-9566 の投与は生存率や症状の改善においてゲムシタビンの効果に劣ることが報告された．

MMP 阻害薬については，抗がん性の分子標的薬剤として未だ臨床応用されるまでには至っていない．がんに特異性の高い MMP 活性化のネットワークシステムを確実に把握することが，今後の MMP を標的とする有効な薬剤の開発の鍵となるであろう．

4. TNP-470

他方，Met AP-2を標的とする薬剤としてフマギリン誘導体であるTNP-470が開発された（表I-4-2）．内皮細胞増殖を特異的に阻害し，血管新生を *in vivo* で阻害する実験結果が示され，動物の移植がんの増殖も著明に抑制することが確認されている．臨床試験が始められているが，その有効性については未だ報告がみられていない．

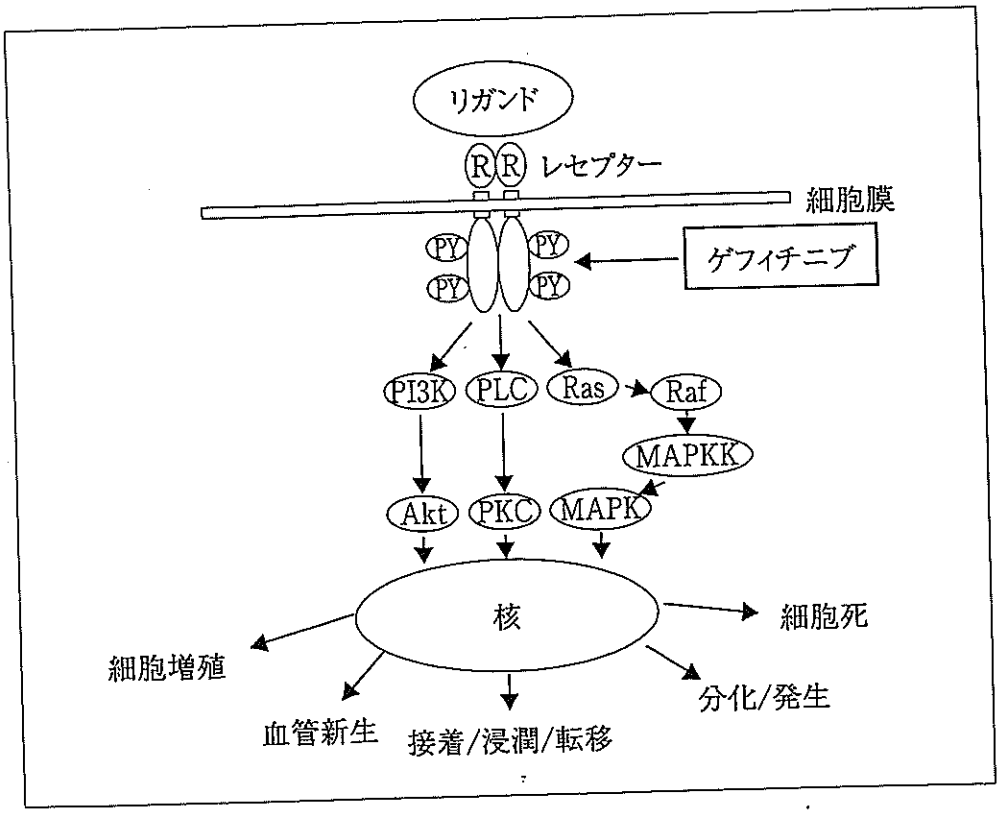
IV. チロシンキナーゼ阻害薬

がん細胞に特異性のある分子をターゲットとした分子標的治療薬の開発は，現在もっとも注目されている．なかでもチロシンキナーゼ阻害薬はすでに多くの分野で臨床効果をあげている[11]．

1. EGFレセプターチロシンキナーゼ特異的阻害薬

EGFファミリー以外にPDGF，IGF，FGF，HGF，VEGFなどはチロシンキナーゼ型レセプターを介して細胞増殖のみならず浸潤，転移，血管新生，細胞死また薬剤感受性などに関連するシグナルを伝達する（**図I-4-2**）．

チロシンキナーゼ型レセプターのerbBファミリーとしてEGFレセプター（erbB 1, HER 1），erbB 2（HER 2/neu），erbB 3（HER 3），およびerbB 4（HER 4）の4種類の蛋白が知られている．これらのレセプターは単量体として存在し，細胞外ドメインに個々に特異的なリガンド結合すると二量体となる．二量体形成によりチロシンキナーゼが活性化され，その結果，チロシンの自己リン酸化が生じ，細胞内蛋白質のチロシン残基もリン酸化され，細胞内へシグナルが伝達される．EGFレセプターを対象として開発されたゲフィチニブ（ZD 1839）[11),12)]は，EGFレセプターのチロシンキナーゼATP結合部位においてATPと競合して，チロシンキナーゼの自己リン酸化を阻害し，EGFレセプターのシグナル伝達を遮断し抗がん作用を示すと考えられている



図Ⅰ-4-2　EGFレセプターのシグナル伝達とゲフィチニブ作用
EGFレセプターはリガンドと反応することにより，さまざまなシグナルを細胞へ伝達する．チロシンキナーゼ阻害薬であるゲフィチニブはこのシグナルを阻害する．PY：phosphotyrosine

(図Ⅰ-4-2)．

EGFレセプターはさまざまなタイプの腫瘍でしばしば過剰発現しており，臨床予後や進展度に相関することが報告されている[11),12)]．現在，ゲフィチニブは切除不能または再発非小細胞肺癌に適応となっている．第Ⅱ相国際共同臨床試験（IDEAL 1）で，プラチナ製剤を含む化学療法による既治療の進行非小細胞肺癌にて対してのゲフィチニブの奏効（CR＋PR）率は18.4%，病勢コントロール（CR＋PR＋SD）率は54.4%と良好な成果をあげている．とくに男性より女性，上皮がんより腺がん，喫煙者より非喫煙者がより有効であるという知見も大変重要である．しかし一方で有害事象としての急性肺障害・間質性肺炎（interstitial lung disease；ILD）が報告されている．2002年7月の承認後，同年12月までの6カ月の間に，推定19,000人以上の肺癌患者に

投与され約1.9%にILDが発症し，約0.6%が死亡したとされる．ゲフィチニブによるILDの発症機序の詳細はわかっていないが，ゲフィチニブ投与後ILDは，早期発症例に死亡例が多く，急激な臨床経過をとることが報告されている．薬剤性の肺障害に対しては通常，副腎皮質ホルモンの投与が有効であるが，ゲフィチニブの場合，抵抗性を示すものも報告されている．ゲフィチニブ投与に際しては十分病態の状況を考慮したうえで，投与を検討すべきである．

2. HER2/neuに対するモノクローナルヒト型化抗体 トラスツズマブ

トラスツズマブ（ハーセプチン®）は分子標的治療薬としてもっとも早く海外で認可を得た薬剤である．現在，日本においても進行・再発乳癌に対して適応となっている．トラスツズマブの作用機序は，免疫細胞を介した抗体依存性細胞障害（antibody dependent cell mediated cytotoxicity；ADCC）や，HER2を介した継続的な細胞増殖シグナル伝達経路の抑制などが考えられているが，詳細は明らかではない[13),14)]．第III相試験においてはトラスツズマブ単独投与群では奏効率17%であったのに対し，化学療法との併用群では41%と著明な改善がみられており，化学療法との併用が積極的に進められている．

3. BCR-ABL特異的チロシンキナーゼ阻害薬

STI-571〔イマチニブ（グリベック®）〕はCMLと，GISTに適応となっている[15)~17)]．イマチニブはBCR-ABLチロシンキナーゼに特異的に作用し，そのリン酸化を阻害する．他方，イマチニブのもう一つの適応疾患であるGISTは消化管にまれに発生する間葉系腫瘍の一種である．大部分がc-Kitを発現し，約60%にc-Kit遺伝子の変異が認められている．KITチロシンキナーゼのATP結合部位にATPと競合的に結合し，KITチロシンキナーゼ活性を選択的に阻害することにより細胞増殖を抑制す

る．2001年のASCOにおいて，CD 117陽性の転移性，もしくは切除不能症例に対し，イマチニブ投与で部分寛解59%，不変26%，進行13%であったと報告されている．また，c-Kitエクソン11，エクソン9，野生型の順に優れた治療効果が観察され，c-Kitの変異部位と奏効率との相関についても報告されている．イマチニブのGIST患者への投与は従来の化学療法や放射線療法と比べ，よりよい抗腫瘍効果が得られている．

以上，チロシンキナーゼを標的とする分子標的薬剤は，従来の殺細胞性抗がん剤に比べ殺細胞が少なくて抗がん効果を示すことが特徴である．

おわりに

いくつかの分子標的薬剤の臨床試験において良い結果が観察され承認されたことは，新しいがんの治療戦略が消化器がんに対しても誕生しつつあることを意味している．とくに，チロシンキナーゼ型レセプターや非チロシンキナーゼ型レセプターの標的薬剤は新しいがん治療戦略を確実に提示しつつある．さらに，ゲフィチニブやトラスツズマブなどの受容体型チロシンキナーゼ標的薬剤について，現在のところ肺癌や乳癌などがおもな対象疾病である．しかし，erb Bファミリーを標的とする分子標的薬剤は，胃癌，大腸癌などの消化器がんおよび前立腺癌や頭頸部腫瘍に対しても臨床試験が進められ大きな期待がよせられている[18),19)]．このことは，分子標的薬剤の開発が消化器がんの治療に貢献するところは大きいと考えられる．

がん治療への応用に際して，

1) いかにして薬剤の分子標的への効果を判定するか

2) いかなる殺細胞性抗がん剤や放射線照射との併用が有効なのか

3) エンドポイントをどう判定するのか

4) 耐性や副作用に対してどう対処するか

など多くの課題を一歩一歩解決することも必要である．がんの悪性形質と関連する分子を標的とした治療薬は，その分子とその生物活性を確かに阻害しているという証明が必要となる（proof of principle）．したがって，がん患者の生体内において目的とする分子標的をモニタリングするシステムを確立することによってproof of principleを具体化することが大切となってくるであろう．

謝辞：貴重な討論をいただいた小野眞弓博士（九州大学医学研究院医化学）と藤井輝彦博士（久留米大学先端癌治療研究センター），原稿をまとめていただいた高木　泉氏（久留米大学先端癌治療研究センター）と津留崎智子氏（久留米大学先端癌治療研究センター）に感謝申し上げます．

文　献

1) 楠本　茂，西條長宏：新しい分子標的治療薬．臨床と研究　80；92-96，2003
2) 小野眞弓，桑野信彦：血管新生とがん．生化学　70；1159-1170，1998
3) 小野眞弓：血管新生抑制剤の開発の現状．佐藤靖史 編：血管新生の最前線．133-147，羊土社，東京，1999
4) 小野眞弓，桑野信彦：血管新生とその阻害剤．現代医療　32；129-135，2000
5) Eider DJ and Paraskeva C：COX-2 inhibitors for colorectal cancer. Nat Med　4；392-393, 1998
6) Steinbach G, Lynch PM, Philips RK, et al：The effect of Celecoxib, acyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med　342；1946-1952, 2000
7) Blanke CD：Celecoxib with chemotherapy in colorectal cancer. Oncology　16；17-21, 2002
8) Kuwano T, Nakano S, Yamamoto H, et al：Cycloxygenase 2 is a key enzyme for inflammatory cytokine induced angiogenesis. FASEB J, 2004 (in press)
9) 曽根三郎，矢野聖二：分子標的転移阻害剤の最新知見．医学のあゆみ　206；271-274，2003
10) Shono T, Kuwano M, Ono M, et al：A new synthetic matrix meallo-proteinase inhibitor modelates both angiogenesis and urokinase type plasminogen activator activity. Angiogenesis　2；319-329, 1999

11) 田村研治，中川和彦，福岡正博：固形癌に対する分子標的治療進歩．医療と宿主　13；344-356，2001
12) 堀池　篤，田村友秀：イレッサによる間質性肺炎とリスク・ベネフィット．Cancer Frontier　15；121-125，2003
13) Slamon DJ, Leyland-Jones B, Shak S, et al：Use of chemotherapy plus a monoclonal antibody against HER 2 for metastatic breast cancer that overexpresses HER 2. N Engl J Med　15；783-792, 2001
14) Pegram MD, Hsu S, Lewis G, et al：Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene　18；2241-2251 1999
15) 大橋明子，廣田誠一：GIST における c-Kit 遺伝子の異常．病理と臨床　20；1556-1560，2002
16) Oosterom AT, Judson I, Verweij J, et al：An active drug in metastatic gastrointestinal stromal tumors (GIST), an EORTC phase I study. Prog Proc Am Soc Clin Oncol　20；2, 2001
17) Oosterom AT, Judson I, Verweij J, et al：Update of the Imatinib (STI 571) phase I study in gastrointestinal stromal tumors (GISTs). Prog Proc Am Soc Clin Oncol　21；82 a, 2002
18) Ranson M：ZD 1839 (Iressa™)：For more than just non-small cell lung cancer. The Oncologist　7 (Suppl 4)；16-24, 2002
19) Daneshmand M, Parolin DAF, Hirte HW, et al：A pharmacodynamic study of the epidermal growth factor receptor tyrosine kinase inhibitor ZD 1839 in metastatic colorectal cancer patients. Clin Cancer Res　9；2457-2464, 2003

（三島麻衣，丸山祐一郎，桑野隆史，桑野信彦）

Review Article

Recent trends in the treatment of advanced lung cancer

Nagahiro Saijo[1]

National Cancer Center, Hospital East, Kashiwanoha 6-5-1, Kashiwa-shi, Chiba 277-8577, Japan

(Received January 27, 2006/Accepted February 13, 2006/Online publication March 31, 2006)

Lung cancer is one of the major causes of death in many countries because of high rates of smoking, especially in Asian countries. Lung cancer is divided into two major categories based on their biological characteristics and the selection of treatment methods: non-small cell lung cancer (NSCLC; 85%) and small cell lung cancer (15%). Early detection and complete resection are very important in NSCLC, but the cure rate is not very high, except in stage 1A disease. It is extremely important to understand the biology of lung cancer and to introduce more effective treatments in order to improve the survival of NSCLC patients. Numerous clinical trials involving lung cancer patients have led to 'state-of-the-art' treatments for each stage of the disease. Progress in chemotherapy and molecular target based therapy have altered the standard therapy for NSCLC. (*Cancer sci* 2006; 97: 448–452)

Chemotherapy for advanced non-small cell lung cancer

Platinum-based doublets are considered to be the standard treatments for stage IV non-small cell lung cancer (NSCLC).[1,2] Although the majority of regimens contain cisplatin, carboplatin can be used in combination with paclitaxel because numerous phase III data exist on this combination. The question remains, however, as to whether or not we can treat advanced NSCLC patients with a non-platinum-based regimen. To date, the answer would appear to be that platinum-based therapy is superior, although platinum drugs and/or non-platinum doublets could be used to treat elderly and/or frail patients because of their low renal toxicity. Kosmidis, the chairman of the Hellenic Cooperative Oncology Group, reported the results of their randomized controlled trials looking at the combination of paclitaxel/gemcitabine versus carboplatin + gemcitabine in advanced NSCLC. More than 500 patients were accrued, of which 445 were evaluative. There was no difference in response rate, time to progression or median survival. There was slightly more hematological toxicity with carboplatin and gemcitabine, although it was relatively mild with only 28% having grade 3 and 4 neutropenia. There was slightly more neurotoxicity in the paclitaxel and gemcitabine arm, and the difference was statistically significant. Kosmidis concluded that this was enough evidence to show that non-platinum-based chemotherapy is as good as platinum-based chemotherapy.[3] However, no studies have demonstrated the superiority of a non-platinum doublet over a platinum-based doublet.

Several doublets that include new drugs improve survival, but no one regimen is clearly superior to the others.[1,2] We have conducted a four-arm cooperative study (FACS) in advanced NSCLC. The study was designed to demonstrate non-inferiority of three experimental arms: paclitaxel + carboplatin; gemcitabine + cisplatin; and navelbine + cisplatin in comparison with cisplatin + CPT-11 (control arm). One-year survival (59%) was higher than expected in cisplatin + CPT-11. No statistically significant differences in response rate, time to progression (TTP) or overall survival were observed between the reference and experimental regimens. Non-inferiority of the three experimental arms was not demonstrated. The response duration in the cisplatin + CPT-11 arm was relatively longer than in the other three arms. No statistical test was conducted because these data were obtained from selected populations based on response, such that there is no statistical basis for comparison (Ohe Y *et al.*, unpublished data, 2006). In conclusion, all four platinum-based doublets have similar efficacy for advanced NSCLC but with different toxicity profiles. Cisplatin + CPT-11 is still regarded as the reference regimen in Japan.

The chemotherapy outcomes were compared in Japanese and American NSCLC patients accrued to the FACS trial and the SWOG 0003 trial,[4] respectively. The two trials had similar eligibility and evaluation criteria, although the dose of paclitaxel was 200 mg/m^2 in the Japanese trial and 225 mg/m^2 in the SWOG trial. The purpose of the analysis was to demonstrate similarities and differences in patient characteristics and outcomes between the Japanese and USA trials for advanced stage NSCLC treated by the same regimen, to provide a basis for standardization of the study design/process to facilitate interpretation of future trials, and to take the first step toward possible joint NCI-sponsored studies in lung cancer between Japanese and American investigators. This analysis using carboplatin and paclitaxel as the common arm shows great similarities in patient characteristics between the FACS trial and the SWOG 0003 trial. Frequencies of neutropenia and febrile neutropenia were significantly higher in FACS trials although the paclitaxel dose was lower

[1]To whom correspondence should be addressed. E-mail: nsaijo@east.ncc.go.jp

doi: 10.1111/j.1349-7006.2006.00198.x

in this group. There may be some differences in population-based pharmacogenomics. Grade 3/4 neuropathy, conversely, was more frequent in the SWOG 0003 trial due to differences in the cumulative paclitaxel dose because of the higher absolute dose and higher median numbers of treatment courses. The response rates were exactly the same, but 1 year survival was better in the FACS trial. These results suggest that future joint Japan–USA clinical trials should consider possible pharmacogenomic differences in drug disposition between Japanese and American populations.[5]

Molecular target-based drugs in advanced recurrent NSCLC

Numerous molecular target-based drugs have been introduced for the treatment of NSCLC, but can they replace current therapy? Can they be used as an adjuvant to current therapy? Can they be combined with other chemotherapeutic agents, radiotherapy and/or surgery?

We hypothesize that incorporation of novel molecular target-based therapies into current treatment paradigms will improve outcomes. However, carefully designed clinical trials and translational science will be required to identify subsets of patients who will benefit.

If we are to use them, we must first answer the following critical questions. Is the target required for a response? Whether or not we know a real and correct molecular target is still questionable. Is the presence of the target sufficient for a response, and can we measure the target in a biologically relevant and/or technologically valid way? Does the agent inhibit the proposed target at the dose and schedule used? Is the target a critical driving force for cell growth in the tumor type in question? The answers to these questions are crucial to treatment with molecular target-based drugs.

Various molecular target-based drugs for advanced NSCLC have been evaluated in randomized controlled trials, but the majority, including a matrix metalloprotease inhibitor, a protein kinase C inhibitor and trastsuzumab, have yielded negative results.[6–8] Gefitinib is an orally available selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor that exhibits antitumor activity in patients with previously treated advanced NSCLC.

Clinical trial of gefitinib and erlotinib

Four open-label multicenter phase I studies have identified diarrhea, skin rash/acne and nausea as common adverse events.[9,10] Two large-scale, multicenter randomized controlled phase II trials, IDEAL 1 and 2, have demonstrated clinically significant antitumor activity of gefitinib monotherapy, and erlotinib has also shown promising antitumor activity.[11] Neither drug showed any additive and/or synergistic effect when combined with platinum-based chemotherapy as a first-line treatment for NSCLC.[12,13]

On December 17, 2004, AstraZeneca announced the preliminary results of their Iressa Survival Evaluation in Lung Cancer (ISEL) study. The study had accrued 1692 patients with advanced recurrent/refractory NSCLC. Unfortunately, Iressa failed to significantly prolong survival compared with a placebo (HR = 0.89, $P = 0.087$) in the overall patient population or among patients with adenocarcinoma (HR = 0.83, $P = 0.089$), although a tendency toward a survival benefit was observed in the gefitinib group.[14] The less than 10% response rate did not result in an overall prolongation of survival. A retrospective analysis of patients treated with gefitinib in clinical practice showed that tumor response predictors included 'adenocarcinoma', 'no history of smoking', 'women', and 'Japanese'. Survival in the gefitinib group in the ISEL study was significantly higher for non-smokers ($P < 0.01$) and Asians ($P < 0.01$) than in the placebo group. The survival curves of the two treatment groups were the same for non-Asians. The data obtained from the ISEL study were not surprising, although most observers had expected positive overall results.

The results of similar randomized trials of erlotinib (BR21) were presented at the American Society Clinical Oncology (ASCO) meeting in 2004. Erlotinib significantly prolonged survival in patients with advanced, previously treated refractory/recurrent NSCLC.[15] The two studies referred to above differed in several respects. Sample size was larger in the ISEL study than in the BR21 study, and 10% of the patients in the latter study had a performance status (PS) of 3, whereas only PS-2 patients were accrued by the ISEL study. The follow-up period of the ISEL study was also relatively short (4 months). The overall percentage of patients with adenocarcinoma and the percentage of non-smokers was 50% and 20%, respectively, in both studies. Data stratification into Asians and non-Asians was only performed in the ISEL study. The stratified survival data for Asians in the BR21, submitted to the US FDA, showed a tendency that was similar to the stratified data in the ISEL study. The survival of non-smokers in the erlotinib group in the BR21 study was extremely good and contributed to the improvement in overall survival in the erlotinib group. How can we explain the discrepancy of the result from the ISEL and BR21 studies? Part of the explanation is that the dose of gefitinib in the ISEL study was low, while the BR21 study used nearly the maximum tolerated dose. Another hypothesis is that patient populations in the ISEL study were inappropriately selected, for example, subjects with poor prognostic factors. The shapes of the survival curves for the Intact 1 and 2, TALENT and TRIBUTE studies and for the non-Asians in the ISEL study suggest that EGFR-TKI does not prolong the survival of non-Asian patients with NSCLC, with or without prior chemotherapy.[12,13,16,17] The stratified survival data of the Asians in the Intact 1 and 2, TALENT and TRIBUTE studies should be analyzed.

In the SWOG 0023 trial, patients with stage III NSCLC received chemoradiation therapy then three cycles of a single agent, docetaxel, followed by either a placebo or gefitinib as maintenance. This trial was projected to have 80% of the patients receiving either placebo or gefitinib with a drop off of 20% during this part of the therapy. The drop off rate before randomization was a bit larger than the expected rate because of progressive disease or death. Investigators asked the Data Safety Monitoring Committee to look at the data to see if they should actually continue the trial because the results of the ISEL study were negative. This early unplanned analysis showed there was no difference in time to progression in either arm and the P-value for difference was 0.54. Similarly, there was no statistically significant difference in

survival and the *P*-value was 0.09, favoring the placebo group. It was surprising and disappointing that the gefitinib-treated patients were actually experiencing worse survival than the placebo patients. This trial had the power to show a 0.33% advantage for gefitinib and the data were sufficient to state that the likelihood of showing a 33% survival improvement was 0.0015.[18] These data suggested that there is no rationale for using gefitinib in locally advanced NSCLC in the adjuvant setting.

Molecular marker predicting clinical outcome of EGFR-TKI

The activities of epidermal growth factor receptor (EGFR) inhibitors, gefitinib and erlotinib in lung cancer and the correlation of responses to somatic mutations are the focus of translational research performed in 2004 and 2005. This answers the major question; which patients respond and why? We have demonstrated that PC-9 cells with a 15 bp deletion in exon 19 of the EGFR gene are extremely sensitive to EGFR-TKI.[19] In April and May 2004, Paez and Lynch reported that activating mutations in EGFR are present in a subset of NSCLC tumors and that the tumors are highly sensitive to gefitinib and erlotinib.[20,21] EGFR expression levels are not a predictor of response and EGFR amplification may have an impact, but EGFR-TK mutations seem to be better predictors of responsiveness to gefitinib and erlotinib.[22–24] Mutant EGFR are more sensitive to ligand stimulation and are dramatically more sensitive to EGFR-TKIs.[19–21] The incidence of EGFR mutations is reportedly higher in Asians, including Japanese,[25,26] and Mitsudomi has reported cumulative percentages of those with EGFR mutation-positive status in 1104 patients with NSCLC to be 34% among Asians and 8% among non-Asians.[27] Eighty percent of the patients who responded to EGFR-TKI carried an EGFR mutation (non-Asians, 79% [30/35]; Japanese, 81%: [39/48]). Among non-responders, 0% of non-Asians and 21% of Japanese patients carried an EGFR mutation. These data suggest that the presence of an EGFR mutation is a strong predictor of a favorable response to EGFR-TKI. Mutations have been reported to be significantly more frequent in women, in patients with adenocarcinoma, and in never smokers, and these findings are consistent with the clinical predictors of tumor response in patients treated with EGFR-TKI. Mitsudomi recently reported that the del 746–750 mutation might be superior to the L858R mutation for predicting the gefitinib response and those patients with EGFR mutations survived longer after the initiation of gefitinib treatment than those without mutations.

Recently it has been demonstrated that an additional mutation at codon 790 induced resistance to originally sensitive mutant cells.[28,29]

A variety of results were presented at the ASCO 2005 meeting in Orland with regards to molecular analysis of the EGFR gene and protein expression in patients accrued to pivotal studies of EGFR-TKIs.[30] Lynch reported the results of an analytical study using resected specimens and biopsy samples obtained during IDEAL and INTACT studies of gefitinib.[31] Patients with either an EGFR mutation or amplification represented distinct populations. Among cases with mutations, large numbers were female, non-smokers, had adenocarcinoma or bronchioloalveolar carcinoma, were Eastern-Asian and often showed dramatic response rates to gefitinib. Because the number of cases for this analysis was not sufficient, it was impossible to draw any conclusions about the impact of mutation and amplification on survival.

Tsao tried to identify certain relations among the response rate and survival and molecular biological features such as the mutation, protein expression and gene copy numbers in the BR21 study conducted by NCI-Canada clinical trial group, which demonstrated that erlotinib does significantly prolong survival as compared with a placebo. Response rates were higher in patients with EGFR mutations, immunohistochemistry (IHC)-positive tumors and high gene copy numbers, but a statistically significant difference was observed for copy numbers only. Survival benefit was greater in patients who were IHC positive and had high gene copy numbers. However, mutation positive patients did not benefit more than mutation negative patients. From these data, Tsao concluded that mutation analysis is not required for the selection of patients who will receive erlotinib.[32]

There are some controversial data on the relationship between biomarkers and clinical outcome.[33–37] One of the reasons for discrepant data is the validity of techniques including the quality of the samples analyzed. Giaccone conducted a cross validation analysis of EGFR mutations in samples obtained from the Free University (the Netherlands) and the Dana Faber Cancer Institute.[38] The results were discrepant in some samples because of poor quality. Another reason is patient selection because it was impossible to obtain samples from all patients with advanced lung cancer. In the retrospective studies reported to date, only a small proportion of patients have had tumor samples evaluable for each biomarker, making patient selection problematic and prone to the introduction of selection bias. It is therefore extremely important that samples be obtained from all patients in studies evaluating the relationships between clinical outcome and biomarkers such as EGFR expression, amplification and mutation. Of course, the techniques for evaluable biomarkers should be valid. In this regard, the report of Takano is most reliable because they analyzed all the samples from all patients using three techniques: IHC, gene copy number and mutation. There were no problems with patient selection. Because they used surgically resected specimens they were able to obtain adequate specimen amounts. It could be concluded that if the analyses were conducted accurately, EGFR mutational status would be the major predictor of outcome and increased EGFR copy number associated with gefitinib sensitivity would significantly depend on the presence of EGFR mutations.[39] Technical innovations are essential for the reproducible and reliable analysis of samples from advanced disease patients because only small amounts of the specimen could be obtained from inoperable lung cancer patients.

EGFR-TKI seems to be a very promising drug for the treatment of East-Asian patients with NSCLC with and without a history of prior chemotherapy. The response rate has ranged from 20% to 33% clinically, and it was 30% in a prospective phase II trial on 100 previously untreated NSCLC patients. The median survival time of the Japanese population in the IDEAL 1 trial was 13.8 months.[11] To date, no survival

doi: 10.1111/j.1349-7006.2006.00198.x

data from a phase III study of gefitinib and erlotinib in East Asia are available because no phase III study has been conducted. However, a randomized controlled trial comparing gefitinib and docetaxel as a second-line treatment is in progress in Japan. The trial has a non-inferiority design and a definitive conclusion will be difficult to obtain. An erlotinib phase II evaluation has just finished the accrual of patients in Japan, but government approval will require more time.

The frequency of EGFR mutations and response rate are higher in East-Asian populations than in Western countries. A global randomized controlled trial is scheduled for comparison of first-line standard platinum-based chemotherapy versus gefitinib in East Asians, non-smokers versus light smokers, and patients with adenocarcinoma.

Bevacizumab

Vascular endothelial growth factor (VEGF) was originally described as vascular permeability factor. VEGF is involved in the regulation of new vessel growth, promotion of the survival of immature vasculature and binding to one of two receptors such as FLT-1 or KDR.[40]

Bevacizumab is a monoclonal antibody against VEGF. It is 93% human, it recognizes all isoforms of VEGF-A and has a prolonged half life which makes it very convenient to administer on an every 2- or 3-week basis.

The preliminary randomized phase II trial of ECOG using 7.5 mg/kg or 15 mg/kg of bevacizumab every 3 weeks did meet its primary objective of improvement in time to progression on the high dose arm; 7.4 versus 4.2 months. Also, response and survival were numerically better. Problems with hemoptysis or pulmonary hemorrhage occurred in six patients (four squamous cell and two adeno), four of which actually proved to be fatal.[41] Based on these experiences, the ECOG 4599 trial was designed. The primary objective was to compare survival and secondary objectives were to look at the response rate, time to progression and toxicity.

Eligibility criteria included non-squamous cell carcinoma, no history of major hemoptysis and of neither thrombotic nor hemorrhagic disorders, and no central nervous system metastasis. Patients received standard dose carboplatin and paclitaxel with or without high dose bevacizumab 15 mg/kg every 3 weeks. The sample size was calculated to be over 842, providing the investigators with 80% power to detect a 25% improvement in median survival time from the usual 8–10 months. ECOG had two planned interim analyses at 286 and 455 deaths. The study was closed after the second interim analysis. Response rate was significantly higher in the bevacizumab arm (27%) versus the control arm (10%). Progression free survival also favored the bevacizmab arm. Overall survival was highly statistically significant; 12.5 months in the bevacizumab arm and 10.2 months in the control arm. The hazard ratio was 0.77.[42] Hemorrhage was more common in the bevacizumab arm with a 45% incidence compared to less than 1% in the control arm. There were eight treatment-related deaths in the bevacizumab arm and two in the control arm. These data lead to the conclusion that bevacizumab improves survival compared to platinum and paclitaxel in patients with non-squamous NSCLC, although a small increase in severe bleeding can be expected. ECOG considers paclitaxel, carboplatin with bevacizumab to be a standard for the treatment of this NSCLC subgroup. The study group suggested some future plans for combining bevacizumab with chemotherapy, radiotherapy and other targeted agents in neoadjuvant or adjuvant settings. In Europe, a clinical trial of bevacizumab combined with cisplatin + gemcitabine is ongoing. The critical question is whether or not they can obtain reproducible positive data even if the chemotherapy regimen is changed from pacltiaxel + carboplatin to cisplatin + gemcitabine. In Japan, a combination phase I/II study of bevacizumab with 5FU + LV or FOLFOX recently completed the accrual of patients. Combination treatment using bevacizumab with paclitaxel + carboplatin is scheduled. How to manage severe bleeding, even in selected populations, and the extremely high cost of bevacizumab will be major issues.

References

1 Schiller JH, Harrington D, Belani CP *et al.* Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002; 346: 92–8.
2 Kelly K, Crowley J, Bunn PA Jr *et al.* Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced NSCLC: a SWOG trial. *J Clin Oncol* 2001; 19: 3210–8.
3 Kosmidis PA, Kalofonos C, Syrigos K *et al.* Paclitaxel and gemcitabine vs carboplatin and gemcitabine, a multicenter, phase III randomized trial in patients with advanced inoperable non-small cell lung cancer. *Proc ASCO* 2005; 23: 621s(7000).
4 Williamson SK, Crowley JJ, Lara PN *et al.* Paclitaxel/carboplatin (PC) vs PC + tirapazamine in advanced NSCLC: A phase III SWOG trial S0003. *J Clin Oncol* 2003; 20: 9097–104.
5 Gandara DR, Ohe Y, Kubota K *et al.* Japan-SWOG common arm analysis of paclitaxel/carboplatin in advanced stage non-small cell lung cancer (NSCLC): a model for prospective comparison of co-operative group trial. *Proc ASCO* 2004; 22: 618s(7007).
6 Shepherd FA, Giaccone G, Seymour L *et al.* Prospective, randomized, double-blind, placebo-controlled trial of marimastat after response to first-line chemotherapy in patients with small-cell lung cancer: a trial of NCI-Canada and EORTC. *J Clin Oncol* 2002; 20: 4434–9.
7 Adjei AA, Mauer A, Bruzek L *et al.* Phase II study of the farnesyl transferase inhibitor R115777 in patients with advanced NSCLC. *J Clin Oncol* 2003; 21: 1760–6.
8 Gatzemeier U, Groth G, Butts C *et al.* Randomized phase II trial of gemcitabine-cisplatin with or without trastuzumab in HER2-positive NSCLC. *Ann Oncol* 2005; 15: 19–27.
9 Ranson M, Hammond LA, Ferry D *et al.* ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. *J Clin Oncol* 2002; 20: 2240–50.
10 Herbst RS, Maddox AM, Rothenberg ML *et al.* Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in NSCLC and other solid tumors: results of a phase I trial. *J Clin Oncol* 2002; 20: 3815–25.
11 Fukuoka M, Yano S, Giaccone G *et al.* Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced NSCLC (The IDEAL 1 Trial). *J Clin Oncol* 2003; 21: 2237–46.
12 Giaccone G, Herbst RS, Manegold C *et al.* Gefitinib in combination with gemcitabine and cisplatin in advanced NSCLC: a phase III trial – INTACT 1. *J Clin Oncol* 2004; 22: 777–84.
13 Herbst RS, Giaccone G, Schiller JH *et al.* Gefitinib in combination with paclitaxel and carboplatin in advanced NSCLC: a phase III trial – NTACT 2. *J Clin Oncol* 2004; 22: 785–94.
14 Thatcher N, Chang A, Parikh P *et al.* Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomized, placebo-controlled, multicentre

study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 2005; 336: 1527–37.
15 Shepherd FA, Pereira J, Ciuleanu TE *et al.* Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005; 353: 123–32.
16 Herbst RS, Prager D, Hermann R *et al.* TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced NSCLC. *J Clin Oncol* 2005; 23: 5892–5899.
17 Gatzemeier U, Pluzanska A, Szczesna A *et al.* Results of a phase III trial of erlotinib (OSI-774) combined with cisplatin and gemcitabine (GC) chemotherapy in advanced NSCLC. *TALENT Trial Proc ASCO* 2004; 22: 619s(7010).
18 Kelly K, Crowley J, Bunn PA Jr *et al.* Preliminary results of S0023: A randomized phase III trial of cisplatin/etoposide/TRT followed by consolidation docetaxelthan maintenance therapy with gefitinib or placebo in patients with locally advanced inoperable stage III NSCLC. *Proceedings ASCO* 2005; 41: No. 7058.
19 Arao T, Fukumoto H, Takeda M *et al.* Small in-frame deletion in the EGFR as a target for ZD6474. *Cancer Res* 2004; 64: 9101–4.
20 Lynch TJ, Bell DW, Sordella R *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004; 350: 2129–39.
21 Paez JG, Jänne PA, Lee JC *et al.* EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* 2004; 304: 1497–500.
22 Han SW, Kim TY, Hwang PG *et al.* Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol* 2005; 23: 2493–501.
23 Johnson BE, Janne PA. Selecting patients for epidermal growth factor receptor inhibitor treatment: a fish story or a tale of mutations. *J Clin Oncol* 2005; 28: 6813–6.
24 Giaccone G, Rodriguez JA. EGFR inhibitors: What have we learned from the treatment of lung cancer? *Nature Clin Prac Oncol* 2005; 2: 554–61.
25 Shigematsu H, Lin L, Takahashi T *et al.* Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005; 97: 339–46.
26 Kosaka T, Yatabe Y, Endoh H *et al.* Mutations of the epidermal growth factor receptor gene in lung cancer: Biological and clinical implications. *Cancer Res* 2004; 64: 8919–23.
27 Mitsudomi T, Kosaka T, Endoh H *et al.* Mutations of the epidermal growth factor receptor gene predict prolonged survival after gefitinib treatment in patients with non-small-cell lung cancer with postoperative recurrence. *J Clin Oncol* 2005; 23: 2513–20.
28 Pao W, Miller VA, Politi KA *et al.* Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005; 2: e73.
29 Kobayashi S, Boggon TJ, Dayaram T *et al.* *EGFR* mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005; 352: 786–92.
30 Marchetti A, Martella C, Felicioni L *et al.* EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 2005; 23: 857–65.
31 Bell DW, Lynch TJ, Haserlat SM *et al.* Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: Molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* 2005; 31: 8081–92.
32 Tsao M-S, Sakurada A, Cutz J-C *et al.* Erlotinib in lung cancer – molecular and clinical predictors of outcome. *N Engl J Med* 2005; 353: 133–44.
33 Cappuzzo F, Hirsch FR, Rossi E *et al.* Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. *J Natl Cancer Inst* 2005; 97: 643–55.
34 Cappuzzo F, Varella-Garcia M, Shigematsu H *et al.* Increased *HER2* gene copy number is associated with response to gefitinib therapy in EGFR-positive non-small cell lung cancer patients. *J Clin Oncol* 2005; 23: 5007–18.
35 Hirsch FR, Varella-Garcia M, Bunn PA Jr *et al.* Epidermal growth factor receptor in non-small-cell lung carcinomas: Correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 2003; 21: 3798–807.
36 Hirsch FR, Varella-Garcia M, McCoy J *et al.* Increased EGFR gene copy number detected by FISH associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. *J Clin Oncol* 2005; 23: 6838–45.
37 Kaye FJ. A curious link between epidermal growth factor receptor amplification and survival: Effect of 'Allele Dilution' on gefitinib sensitivity? *J Natl Cancer Inst* 2005; 97: 621–3.
38 Giaccone G, Janmeat M, Ruiz MG *et al.* EGFR mutations do not accurately predict response to erlotinib in first line monotherapy treatment of advanced non small cell lung cancer. *Lung Cancer* 2005; 49: S244 (P–485).
39 Takano T, Ohe Y, Sakamoto H *et al.* Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. *J Clin Oncol* 2005; 23: 6829–37.
40 Jain RK. Molecular regulation of vessel maturation. *Nat Med* 2003; 9: 685–93.
41 Johnson DH, Fehrenbacher L, Novotny WF *et al.* Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J Clin Oncol* 2004; 22: 2184–91.
42 Sandler AB, Gray R, Brahmer J *et al.* Randomized phase II/III trial of paclitaxel plus carboplatin with or without bevacizumab in patients with advanced non-squamous NSCLC: An Eastern Cooperative Oncology Group Trial-E4599. *Proc ASCO* 2005; 23: 2s (LBA4).

doi: 10.1111/j.1349-7006.2006.00198.x

Lung Cancer (2006) 54, 217–225

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/lungcan

Eg5 expression is closely correlated with the response of advanced non-small cell lung cancer to antimitotic agents combined with platinum chemotherapy

Takamoto Saijo[a,*], Genichiro Ishii[b], Atsushi Ochiai[b], Kiyotaka Yoh[a], Koichi Goto[a], Kanji Nagai[a], Harubumi Kato[c], Yutaka Nishiwaki[a], Nagahiro Saijo[a]

[a] *Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwa, Chiba 277-8577, Japan*
[b] *Pathology Division, National Cancer Center Research Institute East, Kashiwa, Chiba, Japan*
[c] *Division of Thoracic Surgery, Tokyo Medical University School of Medicine, Tokyo, Japan*

Received 28 February 2006; received in revised form 4 June 2006; accepted 22 June 2006

KEYWORDS
Eg5;
Antimitotic agents;
Platinum-based chemotherapy;
Advanced non-small cell lung cancer;
Cyclin B1

Summary
Background: Eg5 is a microtubule motor protein that functions in bipolar spindle assembly. We investigated the relationship between Eg5 expression and the response to chemotherapy of patients with advanced non-small cell lung cancer (NSCLC).
Patients and methods: Eg5 expression was investigated immunohistochemically in 122 formalin-fixed tumor samples from untreated stage IIIB or IV NSCLC patients. We also investigated cyclin B1 expression, which is involved in the G2/M transition. All patients received antimitotic agents combined with platinum chemotherapy. The response to chemotherapy was compared in relation to Eg5 and cyclin B1 expression and in relation to clinicopathological factors.
Results: The response rate to chemotherapy of patients with Eg5-positive tumors was 37%, as opposed to 10% for patients with Eg5-negative tumors, and Eg5 expression was significantly associated with the response to chemotherapy ($P=0.002$). The response rate of patients with cyclin B1-positive tumors (53%) was higher than that of patients with cyclin B1-negative tumors (23%) ($P=0.009$), and Eg5 expression was significantly correlated with cyclin B1 expression ($P=0.005$). A multivariate analysis confirmed Eg5 status to be an independent variable related to response to chemotherapy ($P=0.008$).
Conclusions: Eg5 expression can predict a response to antimitotic agents combined with platinum chemotherapy among patients with advanced NSCLC.

* Corresponding author. Tel.: +81 3 3342 6111; fax: +81 3 3349 0326.
E-mail address: tsaijo@tokyo-med.ac.jp (T. Saijo).

0169-5002/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2006.06.018

1. Introduction

Lung cancer is a major cause of death from cancer worldwide, and non-small cell lung cancer (NSCLC) accounts for ~85% of all cases of lung cancer. More than half of patients with NSCLC have advanced stage IIIB or IV disease at presentation, and patients with advanced NSCLC are candidates for systemic chemotherapy [1]. Meta-analyses have demonstrated that cisplatin-based chemotherapy for metastatic NSCLC statistically improves patient survival, compared with supportive care alone [2]. However, the response rate to chemotherapy has been poor, and very few patients survive for 5 years [3]. During the 1990s, five new drugs became available for the treatment of metastatic NSCLC: paclitaxel, docetaxel, vinorelbine, gemcitabine, and irinotecan. Each of these drugs has since been evaluated in combination regimens with cisplatin or carboplatin and has produced responses in 20–30% of patients [1]. Unfortunately, despite the increasing number of active chemotherapeutic agents, none of these chemotherapeutic regimens has offered a significant advantage over the others in the treatment of advanced NSCLC in randomized studies [4,5], and advanced NSCLC patients still have a median survival time of <1 year. Several reasons have been offered to explain the response to chemotherapy, such as the presence of drug-resistant tumor cells [6] and the redistribution of tumor cells within the cell cycle after chemotherapy. However, the molecular basis of the response to chemotherapy remains to be explored.

A network of microtrabecular filaments forms the cytoplasmic matrix, giving rise to the concept of the cytoskeleton, which comprises microtubules, actin, and intermediate filaments. Microtubules display a remarkable versatility of function and are involved in multiple biologic phenomena, including mitosis, cell shape determination, cell locomotion, and the movement of intracellular organelles [7]. Microtubule-polymerizing agents, including paclitaxel and docetaxel, and microtubule-depolymerizing agents, including vinorelbine, target preliminary tubulin and can induce disrupting kinetic stabilization of microtubules' polymerization–depolymerization, thus blocking the cell cycle in the mitotic phase [8].

Microtubule motors bind to and move unidirectionally on microtubules, and they have been proposed to generate the force required for spindle assembly and maintenance, attachment of the chromosomes to the spindle, and movement of chromosomes toward opposite poles. The microtubule motor proteins, which are members of the kinesin, dynein, or myosin families, can account for many of the movements of the spindle and chromosomes in dividing cells. Kinesin motors have been shown to be necessary to establish spindle bipolarity, position chromosomes on the metaphase plate, and maintain forces in the spindle [9]. Evidence that kinesin motors facilitate microtubule depolymerization also exists, raising the possibility that the motors modulate microtubule dynamics during mitosis. Eg5, which is a part of the kinesin-5 molecule (a member of the kinesin superfamily), is a microtubule motor protein. Eg5 accounts for many of the movements of the spindle and chromosomes in dividing cells and localizes to the spindle in mitotically dividing cells. It has been implicated in spindle function by both its cellular localization and the effects of mutations. Eg5 function in centrosome or spindle pole body separation is necessary for bipolar spindle assembly [10]. The latest antimitotic agent, named monastrol, is an inhibitor of mitotic kinesin Eg5 [11,12]. Monastrol arrests mitosis by reversibly inhibiting mitotic kinesin Eg5 and impairing bipolar mitotic spindle formation. Prolonged mitotic arrest leads to apoptosis in tumor cells and to senescence or apoptosis in primary cells, and the inhibition of mitotic kinesin Eg5 results in the formation of monoaster spindles leading to mitotic arrest [13].

Cyclin and cyclin-dependent kinase complexes play an important role in the control of the cell cycle [14], and the cyclin B1/cdc2 complex has a role as a maturation/mitosis-promoting factor in the G2–M phase transition during the cell cycle [15]. Thus, lack of regulation of cyclin B1 expression may be involved in uncontrolled cell growth and malignant transformation. Overexpression of cyclin B1 has been reported in various malignant tumors and has been shown to predict a poor outcome in NSCLC, esophageal carcinoma, and head and neck cancer [16–18].

In this retrospective study, we investigated the level of expression of Eg5, in addition to cyclin B1—a molecule involved in the G2/M transition, in clinical samples from patients with advanced NSCLC who were subsequently treated with antimitotic agents and investigated whether its expression predicts response to chemotherapy and outcome.

2. Materials and methods

2.1. Subjects

A total of 122 stage IIIB or IV NSCLC patients received platinum-based combination chemotherapy combined with docetaxel, paclitaxel or vinorelbine at the National Cancer Center Hospital East between August 1997 and July 2004 because of PS 0 or 1 on the Eastern Cooperative Oncology Group scale. Adequate tumor biopsy specimens were obtained from all 122 of these patients before chemotherapy and were analyzed in this study. All of the tumor specimens were obtained before chemotherapy, by bronchoscopy in 83 patients, by percutaneous needle biopsy in 31 patients, by thoracotomy in five patients, and by mediastinoscopy in three patients. The histological classification was based on the third edition of the WHO classification. Clinical staging was based on an initial evaluation consisting of a clinical assessment, chest radiography, computed tomography of the chest and abdomen, computed tomography or magnetic resonance imaging of the brain, and bone scintigraphy. The current international staging system was used for clinical disease staging [19]. The clinicopathological characteristics of all the patients are listed in Table 1. Their median age at diagnosis was 62 years (range, 42–78 years). Seven of the 43 stage IIIB patients were women, and 32 of the 79 stage IV patients were women. All of the patients were treated with antimitotic agents combined with platinum chemotherapeutic regimens in what were considered standard regimens for patients with metastatic NSCLC [20]. Nine of the 43 stage IIIB patients received thoracic radiotherapy after the completion of chemotherapy; three of these patients were women. The median follow-up time of the 122 patients was 26 months (range, 18–54 months).

Table 1 Characteristics of 122 patients with advanced NSCLC

Characteristics	No. of patients
Total no. of patients	122
Gender	
Male	83
Female	39
Age (years)	
Median	62
Range	42–78
Histology	
Adenocarcinoma	80
Squamous cell carcinoma	28
Large cell carcinoma	13
Others	1
Stage	
IIIB	43
IV	79
Performance status	
0	32
1	90
Chemotherapeutic regimen	
Cisplatin + vinorelbine	76
Cisplatin + docetaxel	20
Carboplatin + paclitaxel	26
Smoking history	
Positive	91
Negative	31

NSCLC: non-small cell lung cancer.

After obtaining informed consent in accordance with our institution's guidelines, all of the patients underwent a tumor biopsy and chemotherapy.

2.2. Chemotherapy

The platinum-based regimens were vinorelbine (25 mg/m^2) on days 1 and 8 plus cisplatin (80 mg/m^2) on day 1 of a 21-day cycle (76 patients), docetaxel (60 mg/m^2) on day 1 plus cisplatin (80 mg/m^2) on day 1 of a 21-day cycle (20 patients), and paclitaxel (200 mg/m^2 administered over 3 h) on day 1 plus carboplatin (dosed with an area under the curve of 6) on day 1 of a 21-day cycle (26 patients). All of the patients received two or more courses of chemotherapy before the appearance of progressive disease. We used the RECIST guidelines [21] to evaluate the response to chemotherapy. A complete response was defined as the disappearance of all clinically detectable lesions for at least 4 weeks. A partial response required a minimum of a 30% reduction in the greatest diameter of all of the measurable lesions for a minimum of 4 weeks. Progressive disease was defined as the appearance of new lesions or an increase in disease of >20% measured in the same manner as for partial response. All other results were classified as "no change". The response rate was defined as the total of the complete response cases and partial response cases expressed as a percentage of all cases. PFS (progression-free survival) was measured from the start of chemotherapy until the documentation of progressive disease or death.

2.3. Immunohistochemistry

Immunostaining was performed on 4-μm formalin-fixed, paraffin-embedded tissue sections. The slides were deparaffinized in xylene and dehydrated in a graded ethanol series. For antigen retrieval, the slides for cyclin B1 were immersed in 10 mM citric buffer solution (pH 6.0) and the slides for Eg5 were immersed in 1 mM EDTA retrieval fluid (pH 8.0). All of the slides were heated to 95 °C by exposure to microwave irradiation for 20 min. The slides were then cooled for 1 h at room temperature and washed in water and PBS. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 15 min. Non-specific binding was blocked by preincubation with 2% BSA plus 0.1% NaN_3 for 30 min; after draining off the blocking serum, the slides were incubated overnight at 4 °C with anti-Eg5 monoclonal antibody (Clone, 20; Dilution, 1:50; BD Biosciences, NJ, USA) or with anti-cyclin B1 monoclonal antibody (Clone, 7A9; Dilution, 1:20; Novocastra Laboratories, Newcastle upon Tyne, UK). The slides were then washed three times in PBS and incubated with a labeled polymer Envision+ (DAKO, Glostup, Denmark) for 60 min. The chromogen used was 2% 3,3′-diaminobenzidine in 50 mM Tris buffer (pH 7.6) containing 0.3% hydrogen. Slides were counterstained with hematoxylin [22,23]. Normal human lung tissue was used as a positive control.

Eg5 staining was considered positive if the cytoplasm of >10% of the tumor cells stained positive. Cyclin B1 staining was considered positive if the nuclei of >10% of the tumor cells stained positive, because the cyclin B1/cdc2 complex translocates from the cytoplasm into the nucleus during the G2/M transition [24–26]. Thus, the criteria for cyclin B1 positivity used in the present report differed from those used in other reports on non-small cell lung cancer, esophageal carcinoma and head and neck cancer. All of the slides were examined and scored independently by two observers (T.S. and G.I.) who had no knowledge of the patients' clinical data. When the antibody evaluations differed between the observers, the observers discussed the results, with or without re-evaluating the slides, until an agreement was reached.

2.4. Statistical analysis

The correlations between immunohistochemical expression and the clinical variables and response to chemotherapy were evaluated by the χ^2-test or Fisher exact test, as appropriate. PFS was used as a clinical marker for duration of response to chemotherapy. Overall survival was measured from the start of chemotherapy to the date of death from any cause or the date the patient was last known to be alive. Survival curves were estimated using the Kaplan–Meier method, and any differences in PFS and survival between the subgroups were compared by using the log-rank test. The Cox proportional hazards model was used for a multivariate analysis. A multivariate analysis examining the correlation between variables and response to chemotherapy was performed by using logistic regression. P values <0.05 were

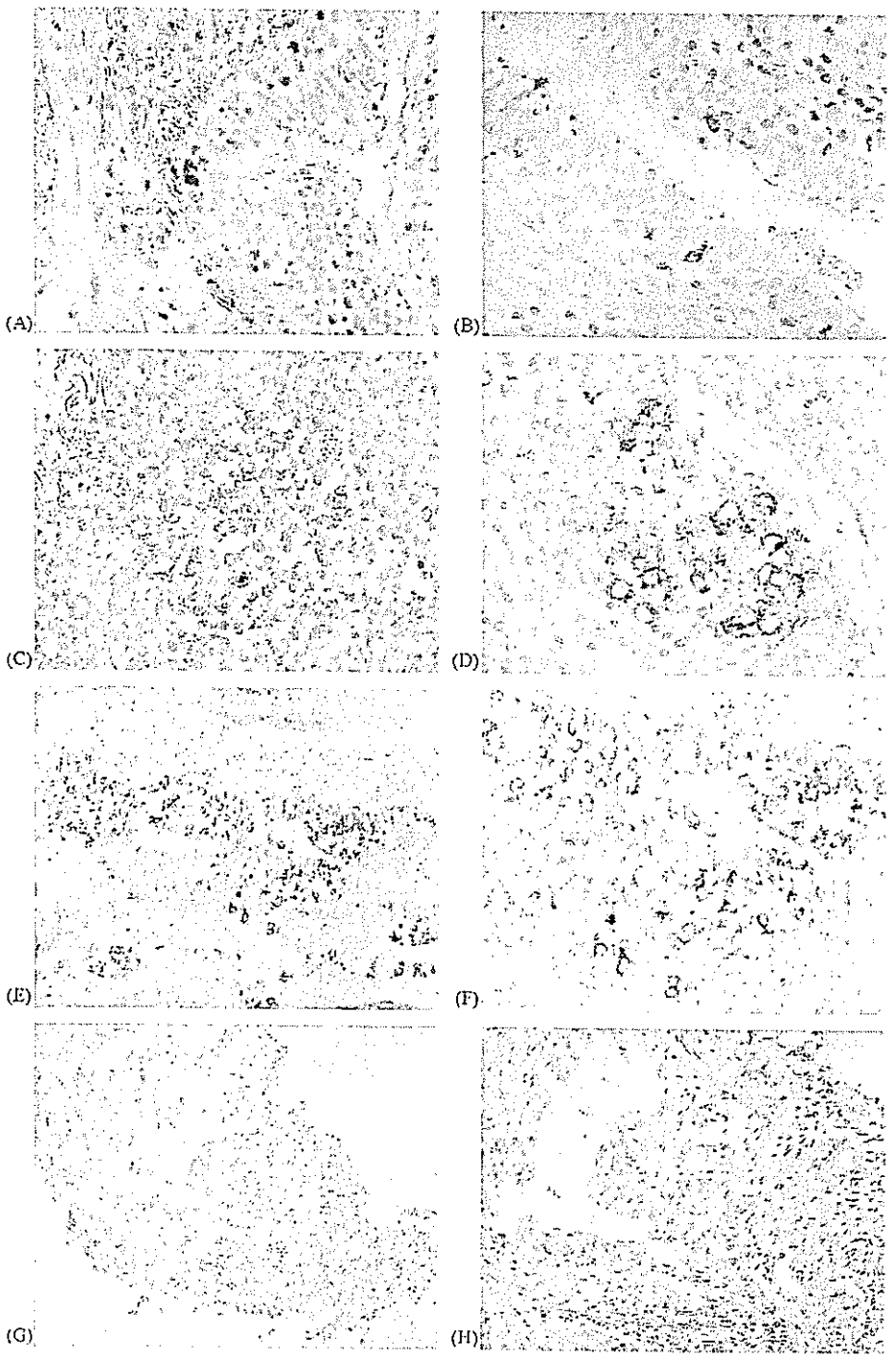


Fig. 1 (A–D) Immunohistochemical staining of Eg5 in normal lung tissue (A), Eg5 is present in part of the basal layer of the bronchial epithelium in this frozen section of normal lung tissue (400×). (B) Eg5 is also present in parts of the basal layer of the bronchial epithelium in this formalin-fixed, paraffin-embedded section of normal lung tissue (400×). (C) Eg5 expression is visible in germinal center lymphocytes giving rise to follicular hyperplasia in this frozen section of normal lung tissue (400×). (D) Eg5 expression is also visible in germinal center lymphocytes giving rise to follicular hyperplasia in this formalin-fixed, paraffin-embedded section of normal lung tissue (400×). (E–H) Immunohistochemical staining of Eg5 in NSCLC (E), low magnification (100×) of squamous cell carcinoma of the lung showing Eg5 immunoreactivity (F), high magnification (200×) of squamous cell carcinoma of the lung showing Eg5 immunoreactivity (G), Eg5 staining was considered to be negative in this adenocarcinoma of the lung: the cytoplasm of <10% of the tumor cells were stained (low magnification; 100×). (H) Eg5 staining was considered to be negative in this adenocarcinoma of the lung: the cytoplasm of <10% of the tumor cells were stained (high magnification; 200×).

considered significant. Two-sided statistical tests were used in all of the analyses. Statistical analysis software (StatView-J Ver. 5.0, Windows) was used for the analyses.

3. Results

3.1. Expression of Eg5 in normal lung tissue

To investigate the validation of immunostaining in the present experiment, we first evaluated Eg5 immunostaining in frozen sections and paraffin-embedded tissue sections of surgical specimens and confirmed that the staining intensity and specificity in the paraffin-embedded tissue sections were almost the same as in the frozen sections. Next, to choose the criteria for immunohistochemical positivity, normal lung tissue was used for Eg5 immunohistochemical staining. Representative immunohistochemical Eg5 staining in normal lung tissue is shown in Fig. 1A–D. In normal lung tissue, Eg5 expression was observed in some of the cells in the basal layer of the bronchial epithelium (Fig. 1A and B) and in germinal center lymphocytes exhibiting follicular hyperplasia (Fig. 1C and D). The frequency of positivity for bronchial epithelial cells and lymphoid germinal center lymphocytes were roughly more than 50% and 90%, respectively. We used these tissues as positive controls. Eg5 immunoreactivity was not detected in the pulmonary parenchyma.

3.2. Expression of Eg5 in NSCLC

The tumors of 82 (67%) of the 122 patients were Eg5 positive. Cytoplasmic staining was observed in most of the Eg5-positive tumors, but some tumors also showed nuclear staining. The median of the percentage staining of the lung cancer cells for Eg5 was 35% (range, 0–100%). Representative immunohistochemical Eg5 staining in NSCLC is shown in Fig. 1E–H. Fig. 1E and F shows the staining results for an Eg5-positive squamous cell carcinoma of the lung. The cytoplasm of almost 80% of the cancer cells stained positive for Eg5. Fig. 1G and H shows an Eg5-negative adenocarcinoma of the lung; this adenocarcinoma of the lung was judged to be negative for Eg5 because the cytoplasm of <10% of the tumor cells showed evidence of staining.

The relationships between the expression of Eg5 and clinical variables are shown in Table 2. Eg5 expression was significantly higher in males than in females ($P=0.03$), in squamous cell carcinoma than in non-squamous cell carcinoma ($P=0.02$), and in current and former smokers than in non-smokers ($P=0.03$).

The tumors of 18 (95%) of the 19 patients with cyclin B1-positive tumors were Eg5 positive, and the tumors of 39 (98%) of the 40 patients with Eg5-negative tumors were cyclin B1-negative (data not shown). Eg5 expression was significantly correlated with cyclin B1 expression ($P=0.005$; data not shown).

3.3. Expression of Eg5 and clinical outcome

All 122 patients were assessed for response to chemotherapy and survival. The relationships between clinical variables, Eg5 expression, and cyclin B1 expression, and the response to chemotherapy and survival in this study are shown in Table 3.

The chemotherapy response rate of patients with Eg5-positive tumors was 37%, as opposed to 10% for patients with Eg5-negative tumors. Eg5 expression was significantly associated with response to chemotherapy ($P=0.002$). The chemotherapy response rate of patients with cyclin B1-positive tumors was 53%, as opposed to 23% for patients

Table 2 Relationship between clinical variables and expression of primary antibodies

	n	Eg5-positive (%) patients	Cyclin B1-positive (%) patients
Total	122	82 (67)	19 (16)
Gender			
Male	83	61 (73)*	15 (18)
Female	39	21 (54)	4 (10)
Histology			
Sq	28	24 (86)**	6 (21)
Non-sq	94	58 (62)	13 (14)
Stage			
IIIB	43	30 (70)	8 19)
IV	79	52 (66)	11 (14)
PS			
0	32	20 (63)	1 (3)
1	90	62 (69)	18 (20)**
Smoking history			
Positive	91	66 (73)*	17 (19)
Negative	31	16 (52)	2 (6)

Sq: squamous; PS: performance status.
* $P=0.03$.
** $P=0.02$.

Table 3 Summary of the relationships between clinical variables and response to chemotherapy and survival

	n	Response rate (%)	*P*	PFS (months)	*P*	MST (months)	*P*
Total	122	28		5.0		12.0	
Gender							
Male	83	28	0.95	5.0	0.43	10.0	0.046
Female	39	28		7.0		15.0	
Histology							
Sq	28	32	0.57	5.0	0.72	9.0	0.64
Non-sq	94	27		5.0		13.0	
Stage							
IIIB	43	33	0.39	6.0	0.01	17.0	0.07
IV	79	25		5.0		11.0	
PS							
0	32	25	0.67	5.0	0.21	14.0	0.16
1	90	29		5.0		10.0	
Smoking history							
Positive	91	27	0.87	5.0	0.23	10.0	0.035
Negative	31	29		6.0		15.0	
Eg5							
Positive	82	37	0.002	5.0	0.08	10.0	0.006
Negative	40	10		6.0		13.0	
Cyclin B1							
Positive	19	53	0.009	5.0	0.77	8.0	0.31
Negative	103	23		5.0		13.0	

PFS: progression-free survival; MST: median survival time.

with cyclin B1-negative tumors, and cyclin B1 expression was also significantly associated with response to chemotherapy ($P=0.009$).

The each of PFS and overall survival curves calculated using the Kaplan–Meier method according to Eg5 expression was shown in Fig. 2. The median PFS time for the Eg5-negative group was 6.0 months, as opposed to 5.0 months for the Eg5-positive group (Fig. 2A). The median survival time for the Eg5-negative group was 13.0 months, as opposed to 10.0 months for the Eg5-positive group (Fig. 2B). According to the overall survival data, the Eg5-positive group had a significantly poorer outcome than the Eg5-negative group ($P=0.006$).

The median PFS time in both the cyclin B1-negative and the cyclin B1-positive group was 5.0 months (Fig. 2C). The median survival time in the cyclin B1-negative group was 13.0 months, as opposed to 8.0 months in the cyclin B1-positive group (Fig. 2D). Cyclin B1 expression was not associated with PFS or overall survival. Among the clinical variables, gender and smoking history were significantly associated with overall survival, and disease stage was significantly associated with PFS, also.

3.4. Multivariate analysis for response to chemotherapy, PFS, and overall survival

Following the univariate analyses for response to chemotherapy, PFS, and overall survival, we performed multivariate analyses. Table 4 shows the results of the multivariate analysis for response to chemotherapy, PFS, and overall survival. The multivariate analysis for response to chemotherapy was performed using logistic regression to determine the prognostic value of Eg5 when other prognostic factors were considered. A multivariate analysis that included gender, histology, stage, PS, smoking history, Eg5 expression and cyclin B1 expression, showed that Eg5 expression was the only significant independent variable correlated with response to chemotherapy ($P=0.008$).

A multivariate analysis using the Cox proportional hazards model for PFS and overall survival was performed, using gender, histology, stage, PS, smoking history, Eg5 expression and cyclin B1 expression, as variables. No correlation between variables and PFS was found in the multivariate analysis. Stage was the only independent variable significantly correlated with overall survival ($P=0.036$).

4. Discussion

This is the study to investigate the relationship between the level of expression of Eg5 and the clinical response to chemotherapy and outcome of previously untreated patients with advanced NSCLC. Eg5, a kinesin motor, accounts for many of the movements of the spindle and chromosomes in dividing cells. It localizes to the spindle in mitotically dividing cells and has been implicated in spindle function by both its cellular localization and the effects of mutations.

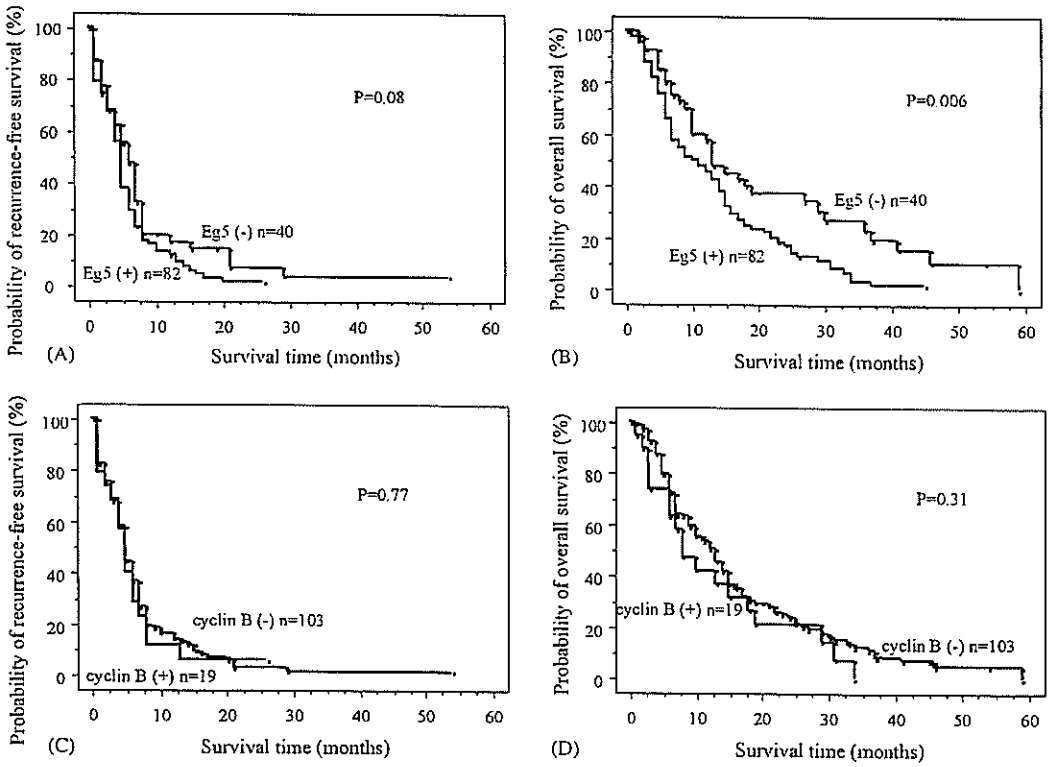


Fig. 2 (A) Progression-free survival curves of 122 patients with advanced non-small cell lung cancer, according to Eg5 expression. The median progression-free survival periods of Eg5-negative and -positive patients were 6.0 and 5.0 months, respectively. (B) Overall survival curves for 122 patients with advanced non-small cell lung cancer, according to Eg5 expression. The median survival periods for Eg5-negative and -positive patients were 13.0 and 10.0 months, respectively. (C) Progression-free survival curves of 122 patients with advanced non-small cell lung cancer, according to cyclin B expression. The median progression-free survival periods of Eg5-negative and -positive patients were 5.0 and 5.0 months, respectively. (D) Overall survival curves for 122 patients with advanced non-small cell lung cancer, according to cyclin B1 expression. The median survival periods for cyclin B1-negative and -positive patients were 13.0 and 8.0 months, respectively.

Eg5 function in centrosome or spindle pole body separation is necessary for bipolar spindle assembly [10].

In normal lung tissue, Eg5 expression was found to be present in some of the cells in the basal bronchial layer of the bronchial epithelium, but its expression in this region was not as strong as in lung cancer tissue. The overexpression of cyclin B1 has been reported in various malignant tumors and has been shown to predict a poor outcome in patients with NSCLC, esophageal carcinoma, and head and neck cancer [16–18]. It has been postulated that the overexpression of cyclin B1 is involved in uncontrolled cell growth and the malignant potential of carcinoma cells. Since the expression of Eg5 in lung cancer tissue has been found to be correlated with the expression of cyclin B1, lung cancer tissue that overexpresses Eg5 in comparison with normal lung tissue is assumed to have greater malignant potential than lung cancer tissue that does not.

Eg5 expression before chemotherapy was correlated with response to chemotherapy and Eg5 status was found to be an independent prognostic factor of response to chemotherapy in a multivariate analysis. Further investigation showed that Eg5 expression was correlated with the response to each type of regimen: the taxan regimens (CDDP + docetaxel: $n = 20$; CBDCA + paclitaxel: $n = 26$; $P = 0.046$), and the vinca alcaroid regimen (CDDP + vinorelbine: $n = 76$; $P = 0.02$) (data not shown). The mechanisms by which Eg5 overexpression affects chemotherapy have not been fully elucidated; nevertheless, Marcus et al. [27] recently reported that mitotic kinesin Eg5 inhibitors induce mitotic arrest and cell death in both paclitaxel-resistant and paclitaxel-sensitive cancer cells and that Eg5 was required for paclitaxel-induced microtubule aster formation (multi-polar spindle configuration) in an *in vitro* assay. They suggested that Eg5 functionality is necessary for paclitaxel-induced mitotic arrest and cell death. These findings may explain our result that Eg5 overexpression before chemotherapy was significantly correlated with response to chemotherapy. The results for docetaxel can be explained in the same manner as for paclitaxel because their modes of action are the same. On the other hand, vinorelbine inhibits the polymerization of tubulin. We suspect that some unknown interaction between tubulin and Eg5 may be modified by vinca alcaloids.

Although Eg5 expression was significantly correlated with response to chemotherapy, the Eg5-positive cases tended to have a poorer outcome in terms of overall survival than the Eg5-negative cases. The reason why the Eg5-positive cases had a poorer outcome remains unclear; despite their higher response to antimitotic agents, Eg5-positive cells may have

Table 4 Multivariate analysis

Variables	Category	Risk ratio	95% CI	P
Multivariate analysis for response of advanced NSCLC patients				
Gender	Male vs. female	0.77	0.245–2.42	0.66
Histology	Sq vs. non-sq	0.89	0.31–2.57	0.83
Stage	IIIB vs. IV	0.64	0.25–1.65	0.35
PS	0 vs. 1	0.98	0.34–2.82	0.97
Smoking history	(−) vs. (+)	0.59	0.18–1.95	0.39
Eg5	(−) vs. (+)	5.16	1.54–17.29	0.008
Cyclin B1	(−) vs. (+)	2.82	0.94–8.45	0.06
Multivariate analysis for PFS of advanced NSCLC patients				
Gender	Male vs. female	0.90	0.56–1.45	0.67
Histology	Sq vs. non-sq	0.89	0.55–1.43	0.63
Stage	IIIB vs. IV	0.60	0.39–0.93	0.02
PS	0 vs. 1	0.92	0.59–1.45	0.72
Smoking history	(−) vs. (+)	0.84	0.51–1.39	0.50
Eg5	(−) vs. (+)	0.77	0.50–1.19	0.24
Cyclin B1	(−) vs. (+)	1.09	0.62–1.89	0.77
Multivariate analysis for OS of advanced NSCLC patients				
Gender	Male vs. female	0.74	0.44–1.26	0.27
Histology	Sq vs. non-sq	1.03	0.63–1.67	0.92
Stage	IIIB vs. IV	0.63	0.41–0.98	0.04
PS	0 vs. 1	0.76	0.47–1.22	0.25
Smoking history	(−) vs. (+)	0.74	0.43–1.30	0.30
Eg5	(−) vs. (+)	0.62	0.39–0.97	0.04
Cyclin B1	(−) vs. (+)	1.03	0.59–1.78	0.93

PFS: progression-free survival; NSCLC: non-small cell lung cancer; PS: performance status; CI: confidence interval; OS: overall survival.

a higher malignant potential, contributing to a poor clinical outcome. This appears to be consistent with the expression of Eg5 being significantly correlated with the expression of cyclin B1, which may be involved in uncontrolled cell growth and the malignant potential of cancer cells.

The inhibition of Eg5 has recently been exploited as an aid to cancer treatment [12–14,27–32], and small cell-permeable molecules that inhibit mitotic kinesin Eg5 and do not target tubulin arrest cells in mitosis with monoastral spindles. Chromosomes in Eg5 inhibitor-treated cells frequently have both sister kinetochores attached to microtubules extending to the center of the monoaster. The mitotic kinesin Eg5 inhibitor also induces apoptosis and is effective in inhibiting the proliferation of cancer cells through mitotic arrest. The first small molecule inhibitor of Eg5 was monastrol [11,12], and second-generation Eg5 inhibitors like CK0106023 [29] and HR22C16 [27], which are specific allosteric inhibitors of Eg5 and exhibit antitumor activity *in vivo* or *in vitro*, have been discovered by drug screens. Therapeutic intervention with Eg5-specific inhibitors has also been reported, and SB-715992 has been shown to be a potent inhibitor of mitotic kinesin Eg5. Eg5 inhibitors may be used as new antimitotic agents to treat advanced NSCLC in the future.

In conclusion, our findings indicated that the expression of the mitotic kinesin Eg5 can predict a response to antimitotic agents combined with platinum chemotherapy among patients with advanced NSCLC. Our results have important implications for the treatment of NSCLC because Eg5 inhibitors, which cause tumor cell apoptosis, may be effective in patients with advanced NSCLC.

Acknowledgements

The authors thank Fumiko Koh for performing data management and Yukinori Murata for providing technical assistance.

References

[1] Bunn Jr PA, Kelly K. New combinations in the treatment of lung cancer: a time for optimism. Chest 2000;117:138S–43S.
[2] Non Small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomized clinical trials. BMJ 1995;311:899–909.
[3] Bunn Jr PA. Chemotherapy for advanced non-small-cell lung cancer: who, what, when, why? J Clin Oncol 2002;20:23S–33S.
[4] Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92–8.
[5] Kelly K, Crowley J, Bunn Jr PA, et al. Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non-small-cell lung cancer: a Southwest Oncology Group trial. J Clin Oncol 2001;19:3210–8.
[6] Mountain CF. Revisions in the international system for staging lung cancer. Chest 1997;111:1710–7.