The place of targeted therapies in the management of non-small cell bronchial carcinoma

Molecular markers as predictors of tumor response and survival in lung cancer

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Résumé
Cette revue souligne les nombreuses découvertes en biologie moléculaire dans le cancer bronchique ayant un impact thérapeutique potentiel aussi bien dans un proche que dans un lointain avenir. De nombreuses données précliniques et cliniques montrent que le taux d’ARNm de BRCA1 est un modulateur différentiel de la sensibilité à la chimiothérapie. De faibles taux sont prédicifs d’une sensibilité au cisplatine et d’une résistance aux agents anti-microtubules et l’inverse est observé pour des taux élevés. Une grande partie des travaux récents a porté sur les mutations et l’amplification du gène EGFR (epidermal growth factor receptor). Ces mutations sont prédicitives de réponse aux inhibiteurs tyrosine-kinase de EGFR chez les patients porteurs d’adénocarcinomes bronchiques métastasés, avec augmentation par trois du temps jusqu’à progression et de la survie pour les patients recevant ce type de molécules. Des preuves s’accumulent quant à l’interaction des voies de signalisation d’EGFR et des œstrogènes, incitant à programmer des essais cliniques associant inhibiteurs tyrosine-kinase d’EGFR et anti-aromatases. La compréhension de l’importance de ces découvertes peut aider à modifier la pratique clinique en cancérologie en adaptant les chimiothérapies et/ou les biothérapies ciblées, et permettant ainsi d’améliorer aussi bien la survie que le coût-efficacité.

Mots-clés : BRCA1 • Mutations EGFR • Résistance aux inhibiteurs tyrosine-kinase • Résistance à la chimiothérapie • Récepteurs aux œstrogènes.
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Summary
This review highlights the numerous molecular biology findings in the field of lung cancer with potential therapeutic impact in both the near and distant future. Abundant pre-clinical and clinical data indicate that BRCA1 mRNA expression is a differential modulator of chemotherapy sensitivity. Low levels predict cisplatin sensitivity and antimicrotubule drug resistance, and the opposite occurs with high levels. The main core of recent research has centered on epidermal growth factor receptor (EGFR) mutations and gene copy numbers. For the first time, EGFR mutations have been shown to predict dramatic responses in metastatic lung adenocarcinomas, with a threefold increase in time to progression and survival in patients receiving EGFR tyrosine-kinase inhibitors. Evidence has also been accumulated on the crosstalk between estrogen and EGFR receptor pathways, paving the way for clinical trials of EGFR tyrosine-kinase inhibitors plus aromatase inhibitors. Understanding the relevance of these findings can help to change the clinical practice in oncology towards customizing chemotherapy and targeted therapies, leading to improvement both in survival and in cost-effectiveness.

Key-words: BRCA1 mRNA • EGFR tyrosine-kinase mutations • Acquired resistance to tyrosine-kinase inhibitors • Estrogen receptors.

Introduction
Chemotherapy in non-small-cell lung cancer (NSCLC) has reached a plateau, with no evidence of substantial improvement in survival. Performance status of 0 is the most significant prognostic factor, though excision repair cross-complementing 1 (ERCC1) mRNA expression is closely linked to cisplatin resistance. The economic impact of novel targeted therapies has not yet been evaluated, and their overall benefit is still meager. Activating mutations in tyrosine-kinases have emerged as a new paradigm for predicting response and outcome. Growing evidence indicates that EGFR deletions and L858R mutations are strong predictors of dramatic responses to gefitinib and erlotinib. Relevant findings in lung cancer molecular biology can pave the way for individualized treatment based on predictive markers; this approach will also contribute to optimizing the cost-effectiveness of novel targeted therapies.

Predictive markers for customized chemotherapy

Between 1993 and 1999, 1436 patients with stage IV or IIIB NSCLC with effusion were treated with platinum-based doublets (involving either paclitaxel, docetaxel, vinorelbine or gemcitabine). The response rates and median survival times were 20% and 8.2 months. One- and two-year survivals were 33% and 11%, respectively [1]. In a multivariate analysis, lower performance status (PS) – PS 1 vs 0 – was identified as one prognostic factor. The salient finding of the Eastern Cooperative Oncology Group (ECOG) trial was that no survival differences were observed between any of the different platinum-based doublets, with a modest benefit for chemotherapy in NSCLC. However, reality shows that survival can vary significantly between individual patients with some surviving years and others succumbing to their disease within a few months.

Cisplatin resistance is associated with increased expression of the ERCC1 gene. Cancer tissues from ovarian cancer patients whose tumors were clinically resistant to therapy showed greater levels of ERCC1 mRNA [2]. We carried out a study to examine the role of ERCC1 mRNA levels in advanced NSCLC patients treated with gemcitabine plus cisplatin. Patients with low ERCC1 mRNA levels attained a response rate of 52%, while those with high levels, the response rate was 36%. This difference was not significant; however, when we used a cutoff of 5.8 for ERCC1 expression, median survival was 15 months for patients with low levels and only 5 months for those with high levels (p<0.001) [3]. Based on these findings, we carried out an ERCC1 mRNA customized chemotherapy trial. More than 400 patients have been included and randomized to the control or the experimental arm. The control arm received docetaxel plus cisplatin, and patients in the experimental arm received either the same combination of docetaxel plus cisplatin if their ERCC1 mRNA levels were low or docetaxel plus gemcitabine if their levels were high (fig. 1). The preliminary results on 264 patients...
[4] showed that the response rate for patients with low ERCC1 levels was 56.6% while for patients in the control arm it was 40.4% (p=0.02). When patients in the control arm were split according to the ERCC1 levels, those with low levels had a response rate of 47.3%, while those with high levels had a response rate of 26.1%. The logistic regression model for tumor progression indicated a significant improvement for patients randomized to docetaxel plus cisplatin based on low ERCC1 levels. Although the results are still preliminary, time to progression and survival adjusted for age are significantly in favor of the group with low ERCC1 levels.

**BRCA1 levels and cisplatin resistance**

BRCA1 was overexpressed in the cisplatin-resistant breast cancer cell line MCF-7 [5]. BRCA1 is component of multiple DNA repair pathways and functions as a molecular determinant of response to a range of cytotoxic chemotherapeutic agents. It has been demonstrated that BRCA1 abrogates the apoptotic phenotype induced by a range of DNA-damaging agents, including cisplatin, etoposide and bleomycin, and induces dramatic responses to a range of antimicrotubule agents, including paclitaxel and vinorelbine. These landmark findings indicate that BRCA1 functions as a differential regulator of chemotherapy-induced apoptosis [6]. Sporadic cancers, such as breast, ovarian and NSCLC, can have the BRCA1 function abrogated by methylation or other mechanisms. These characteristics, known as “BRCAness”, increase sensitivity to cisplatin and related DNA cross-linking agents and may increase resistance to antimicrotubule drugs [7]. Recently, it has been shown that BRCA1 or BRCA1 dysfunction profoundly sensitizes cells to the inhibition of poly(ADP-ribose) polymerase (PARP) [8]. We have observed that BRCA1 mRNA expression closely correlates with ERCC1 mRNA expression and that BRCA1 mRNA expression predicts outcome in locally advanced NSCLC patients treated with neoadjuvant gemcitabine plus cisplatin followed by surgery. Median survival has not been reached in patients with the lowest BRCA1 mRNA levels, while survival was very poor in patients with the highest levels [9]. These findings are along the same lines as preclinical data, indicating that patients with high BRCA1 levels could respond more favorably, not to non-cisplatin regimens but to antimicrotubule drugs. Although gemcitabine is a neutral drug for the BRCA1 mRNA effect, we have observed that elevation of ERCC1 and BRCA1 is closely related to high levels of ribonucleotide reductase, which is one of the principal mechanisms of resistance to gemcitabine [10]. With the exception of ribonucleotide reductase, the mechanisms of gemcitabine resistance have not been explored in the clinical setting. Experimental evidence indicates that increased expression levels of human equilibrative nucleoside transporter 1 (hENT1) are associated with gemcitabine sensitivity while decreased levels of deoxycytidine kinase (dCK) predict acquired resistance to gemcitabine in NSCLC cells [11].

**Gene mutations as predictive markers for molecular therapy**

Inhibition of tyrosine-kinase receptors (TKRs) by selective small molecule inhibitors is emerging as a new strategy for treatment of hematologic malignancies and solid tumors, including leukemias, gastrointestinal stromal cell tumors and NSCLC. Determination of EGFR expression is not sufficient to predict sensitivity to EGFR TK inhibitors. The identification of somatic mutations in the TK domain of the EGFR gene represents the most important molecular marker of sensitivity to EGFR TK inhibitors [12-14]. In a recent study, neither EGFR nor p-EGFR (phosphorylated-EGFR) protein expression was correlated with gefitinib response in chemorefractory NSCLC patients. Expression of downstream markers, like p-Erk, was negatively associated with response. Those without p-Erk staining had a response rate of 38% and those with 1+ staining had 14%, while there was no responder among patients with 2+ staining. Furthermore, tumors with positive p-Akt and negative p-erkr nuclear expression exhibited the best response (60%). Patients with positive p-Akt tended to show prolonged time to progression (6.8 versus 2.5 months; p=0.05) and significantly prolonged survival, regardless of p-Erk expression. The authors speculate that tumors with PI3K/Akt as a preferential downstream pathway are more susceptible to gefitinib whereas those with Ras/Raf/Erk are more resistant [15].

Intriguingly, gefitinib-resistant adenocarcinoma cell line populations have increased Akt phosphorylation (not inhibited by gefitinib), reduced PTEN protein expression and loss of the EGFR gene mutation, when compared with parental cell lines [16].

Second-line erlotinib has yielded a response rate of 8.9%, in contrast with less than 1% in the placebo group. Progression-free survival was 2.2 months versus 1.8 months, with median survival 6.7 versus 4.7 months. Responses were higher in females, adenocarcinomas and never-smokers. There were no differences according to EGFR expression in the total of 731 patients examined [17]. However, in a multivariate analysis of the same study, EGFR expression by immunostaining was associated with better response [18].

In addition, amplification or high polysomy of the EGFR gene (seen in 33 of 102 patients treated with gefitinib) and high protein expression (seen in 58 of 98 patients) were significantly associated with better response (36% versus 3%; p<0.001), time to progression (9 versus 2.5 months; p=0.001) and survival (18.7 versus 7 months; p=0.03). EGFR mutations (seen in 15 of 89 patients) were also significantly related to response and time to progression, but the association with survi-
val was not statistically significant, and 40% of patients with mutations had progressive disease. In the multivariate analysis, only high EGFR gene copy number remained significantly associated with better survival [19]. EGFR mutations have been examined in 68 gefitinib-treated chemorefractory NSCLC patients from the United States, Europe and Asia. Responses were observed in 94% of patients harboring EGFR mutations, in contrast with 12.6% with wild-type EGFR (p<0.001) [20]. These results mirror accumulated data from the three seminal studies of EGFR mutations, in which 81% of NSCLC patients with EGFR mutations attained an objective response whereas none of 29 non-responders had mutations. In the Taron et al study [20], patients harboring EGFR mutations attained dramatic and durable responses with disappearance of brain metastases and other metastatic lesions. Only 16% of responders with wild-type EGFR, compared to 81% of responders with EGFR mutations, are still alive, and median survival has not been reached for this group. In a sub-group of patients, the response rate for patients with increased gene copy numbers was 45%, in contrast with 89% for patients with EGFR mutations (p=0.02). The response rate was 100% for patients with both increased gene copy numbers and EGFR mutations. Interestingly, patients with both EGFR mutations and low levels of EGFR or caveolin-1 mRNA had a median survival of 13 months, whereas median survival has not been reached for those patients with EGFR mutations and high levels of EGFR or caveolin-1 mRNA [20]. Another study has confirmed the low rate and short duration of response in Spanish second-line gefitinib-treated patients with wild-type EGFR [21]. Time to progression for patients with mutations was 12.3 months, compared to 3.6 months for those with wild-type EGFR (p=0.002). In all studies of EGFR mutations to date [20-28], the majority of the mutations were in-frame deletions in exon 19 and the missense mutation L858R in exon 21. Only one study examined the role of EGFR mutations in patients treated with first-line gefitinib [22]. Seventeen patients (19%) harboring EGFR mutations had an impressive time to progression of 21.7 months, in contrast with 1.8 months for those without mutations (p<0.001). Median survival was a landmark 30.5 months for patients with mutations in comparison with 6.6 months for those with wild-type EGFR (P<0.001). P-Akt expression was not associated with response, time to progression or survival [22].

Table 1 shows recent salient findings with regard to EGFR mutations. Some evidence suggests that deletions can predict better response than the missense mutation L858R [23]. In addition, other studies report that the L858R mutation is more frequent in women than in men [24,25]. Intriguingly, squamous cell carcinoma patients with EGFR mutations did not respond to gefitinib treatment [26]. The lowest frequency was found in African-American patients [27], and the different frequencies found between patients from Maryland (6.3%) and Minnesota (20.3%) can be attributed to the higher proportion of African-Americans from Maryland [27].

A large-scale study of EGFR mutations was initiated by the Spanish Lung Cancer Group in April 2005; in the first year, tumors from more than 1000 patients were examined, with striking differences in frequencies according to smoker status (4% in active smokers, 10% in ex-smokers, and 40% in never-smokers).

In a study of paclitaxel plus carboplatin with or without erlotinib (TRIBUTE) [28], the influence of EGFR mutations was examined in 274 patients. Patients with EGFR-mutant tumors in the erlotinib arm attained better outcome than those receiving chemotherapy alone (response: 53% versus 21%; time to progression: 12.5 versus 6.6 months, respectively). A detrimental effect of K-ras mutations was observed for patients in the erlotinib arm, with a response rate of 8%, compared to 23% for those receiving chemotherapy alone. Time to progression and survival were also shorter for patients with K-ras mutations receiving erlotinib than for those receiving chemotherapy alone (time to progression: 3.4 versus 6 months; survival: 4.4 versus 13.5 months) [28]. Numerous EGFR mutations have also been found in sporadic and hereditary breast cancers, in the form of point mutations; neither deletions nor the L858R mutation were observed [29]. Mutations were found more frequently in hereditary breast cancer than in sporadic breast cancer, which can be attributed to genomic instability stemming from disruption of DNA repair capacity by defects in BRCA1 and BRCA2 [29]. This raises the hypothesis that the better survival observed with chemotherapy alone in patients with EGFR mutations [28] may be due to the underlying DNA repair defects that can accompany EGFR mutations.

Mutations in other TKs have also been described, including ERBB2 mutations, originally found in 5 of 51 lung adenocarcinomas (10%) [30]. Nevertheless, ERBB2 mutations were found at a much lower frequency: 11 of 671 NSCLCs (1.6%). All mutations were in-frame insertions in exon 20 and were more frequent in never-smokers (3.2%) and in adenocarcinomas (2.8%) [31]. No ERBB2 mutations were found in Korean lung adenocarcinoma patients [32].
Activating mutations of platelet-derived growth factor receptor α (PDGFRα) have been found in gastrointestinal stromal tumors with wild-type KIT [33]. These mutations could be found in other tumors that are sensitive to imatinib and other small molecule drugs that inhibit PDGFRα kinase activity. PDGFRα activation loop (exon 18) mutations have been identified in only one of 45 NSCLCs examined (Santarpia M, personal communication).

PI3K3CA missense mutations in exons 9 and 20 were found in one of 24 lung cancers (4%) [34]. Recently, PI3K3CA mutations were not found in any of 100 NSCLCs (Santarpia M, personal communication).

**Estrogen and progesterone receptors in NSCLC**

It is well-known that a significant number of NSCLCs overexpress estrogen and progesterone receptors and that cross-talk between estrogen receptors and EGFR pathways is common. Experimentally, it has been demonstrated that EGFR protein expression was downregulated in response to estrogen and upregulated in response to fulvestrant (an estrogen receptor antagonist), suggesting that the EGFR pathway is activated when estrogen is depleted in NSCLC [35]. This important finding paves the way for clinical trials of EGFR TK inhibitors with aromatase inhibitors.

Progesterone receptor immunoreactivity was observed in 46.5% of 228 NSCLC patients and was associated with better clinical outcome. Positive progesterone receptor status was more frequently seen in males, stage I disease and adenocarcinomas [36].

**Conclusions**

Several layers of evidence indicate that multiple genetic disturbances found in human cancer cell lines and in the tumors of NSCLC patients can be incorporated as predictive markers for response and improved survival with chemotherapy regimens currently used. These markers can also be used to identify subgroups of patients that can have a dramatic response when treated with novel targeted therapies. The paradigm is the impressive response to gefitinib or erlotinib in the presence of EGFR TK mutations – responses two or three times greater than what can be attained with chemotherapy. The use of rapid and sensitive PCR assays for diagnostic screening, coupled with a greater accessibility to tumor tissue from lung cancer patients, will help facilitate the comprehensive application of this knowledge and could lead to improved survival and optimal use of health resources. Along these lines, the Spanish Lung Cancer Group has undertaken a large-scale study in which EGFR mutations are being examined in newly-diagnosed lung adenocarcinoma patients; those harboring EGFR mutations receive erlotinib, and those with wild-type EGFR receive customized chemotherapy.

**Acknowledgements** – Research supported in part by Spanish Ministry of Health grants provided through Red Temática de Investigación Cooperativa de Centros de Cáncer (CO-010), by La Fundación Badalona Contra el Cáncer, and by La Fundación Carvajal. The authors thank Renée O’Brate for assistance with the manuscript and Lourdes Franquet for technical assistance.

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