Published in final edited form as:
Biomark Med. 2008 December 1; 2(6): 577–586. doi:10.2217/17520363.2.6.577.

Biomarkers in lung cancer: from early detection to novel therapeutics and decision making

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Abstract

Lung cancer remains a significant cause of mortality worldwide. While advances in therapy continue to be made, the overall prognosis for patients diagnosed with lung cancer remains poor. Historically, markers such as age, performance status and disease stage have been used to risk-stratify patients and guide therapeutic decisions. These parameters provide some useful information, but more sensitive markers are clearly needed. Molecular and genetic studies have identified several such markers, which appear to play critical roles in carcinogenesis and affect patient outcomes. This article reviews a number of biomarkers that have been identified in lung cancer, and their prognostic and predictive roles.

Keywords

biomarkers; lung cancer; predictive markers; prognostic markers

Despite significant advances in therapy, lung cancer remains the leading cause of cancer-related mortality in the US A, with 161,840 estimated deaths in 2008 [1]. Traditional prognostic markers such as age, sex, performance status, weight loss and other comorbidities have provided some information in predicting how a patient will respond to therapy. However, even in patients with early-stage disease, a significant number will recur and ultimately die of their disease, with the 5-year overall survival for patients with lung cancer remaining at approximately 15%. A number of different markers have been evaluated in patients with lung cancer, from those aimed at early disease detection, assigning prognosis and detecting minimal residual disease, to those predicting both rates of relapse and response to treatment. These include serum markers as well as molecular and genetic markers, and it is only recently that they are beginning to be integrated into clinical decision making. With the development of proteomics and molecular assays, it has been possible to identify additional unique markers that may have prognostic significance and can help influence clinical decisions, from diagnosis to treatment planning. We will review some of the markers identified in lung cancer, and their predictive roles. Where possible, we have referred to other reviews as well as recently published trials on this vast, expanding subject, using sources appearing in PubMed over the last few decades (1968–2008).

Serum markers

A number of serum tumor markers have been studied in lung cancer, including carcino emryonic antigen (CEA), CA-125, CYFR A 21–21, chromogranin A, neuron-specific enolase

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(NSE), retinol-binding protein (RBP), α1-antitrypsin and squamous cell carcinoma antigen. However, no single blood test exists for lung cancer. CEA has been a widely studied tumor marker, and it has been reported that it is elevated in 0–38% of small cell lung cancer (SCLC) patients with limited disease and in 40–65% of those with extensive disease. It is estimated that CEA is elevated in 30–65% of patients with non-small-cell lung cancer (NSCLC). In a retrospective study of 153 NSCLC patients whose tumors were completely resected, Muley *et al.* found that patients who had an elevated CEA or CYFRA 21–21 level had lower overall survival rates than patients with normal levels [2]. In patients with stage IA disease, preoperative CEA levels above 5 ng/ml correlated with a poorer disease-free survival (22.2 vs 75%). Although this may identify a subset of early-stage patients who should be treated more aggressively, the number of patients who fall into this category is relatively small [3]. Both CEA and CA-125 appear to be lower in patients with early-stage disease compared with those with metastatic disease, and in a study that included 37 patients with advanced NSCLC, a decrease in these markers was found in those who had a documented radiologic response [4].

The prognosis in patients diagnosed with SCLC remains dismal. This is, in part, due to the aggressive nature of the disease, coupled with an often advanced stage at diagnosis and high propensity for relapse. An elevated lactate de hydrogenase level has been shown to be a negative prognostic marker in SCLC [5,6]. NSE is thought to be a sensitive marker at the time of diagnosis, although its prognostic value remains controversial. A number of studies have shown this marker to be elevated in patients with SCLC [7–9]. In addition, a rise in NSE levels after an initial fall during chemotherapy has been shown to correspond to a significantly shorter duration of remission [8]. In one study, patients who had a normal serum NSE value at day 28 had a higher complete response rate and overall survival when compared with patients with elevated levels [9].

Since more advanced stages of lung cancer are incurable, early detection is essential, although no screening test currently exists. A recent study reported on a panel of serum biomarkers to aid in the diagnosis of lung cancer: CEA, RBP and a1 antitrypsin. It was found that expression of these proteins had a sensitivity of 89.3% and specificity of 84.7% in correctly identifying patients with lung cancer. They could potentially be used to plan the management of a patient with a pulmonary lesion or as a screening tool in high-risk populations [10].

Molecular markers

While a number of potential biomarkers have been identified, their clinical utility remains largely limited. The data are often retrospective, and larger prospective trials are needed [11, 12]. Given the conflicting data and lack of a serum biomarker that consistently predicts prognosis, additional efforts have focused on attempting to identify new biomarkers through proteomics. Although there has been a great deal of focus on genomics, it is now recognized that proteomics (defined as the study of the proteome, or all of the cellular proteins in a biological entity) serves as a platform for a more comprehensive understanding of carcinogenesis. While genomics has played a critical role in our understanding of cancer pathophysiology, it is limited by the fact that the functional significance of post-translational modifications in various genes may not be detected. Proteomics allows for the detection of these modifications and their impact on carcinogenesis. 2D gel electrophoresis has been used in the past to identify potential biomarkers in both NSCLC and SCLC; however, the process is cumbersome and offers only limited sensitivity [13,14]. Another limitation in identifying potential markers in lung cancer is the limited amount of tissue that is often available. For this reason, the identification of a serum biomarker would be of great clinical utility. Plasma proteome analysis is a complex undertaking, and great advances have been made in the past few years. Using multidimensional chromatography and mass spectrometry (MS) analysis, 1175 unique plasma proteins have been identified [15]. Other techniques have also been

developed, including direct tissue MS. Proteomic patterns have been identified that have allowed investigators to differentiate NSCLC histology, as well as to identify patients with poorer prognosis in resected NSCLC [16]. Additional approaches include surface enhanced laser desorption ionization time-of-flight, which has been used by Xiao *et al*. to identify serum bio markers for early detection of lung cancer. A model was constructed by comparing the serum of 30 patients with lung cancer with aged-matched controls. When tested on blinded serum samples of 15 patients with lung cancer, the test yielded a sensitivity and specificity of 93.3 and 96.7%, respectively. [17] Whether derived from serum or directly from tissue, the samples are incredibly complex, and protein–antibody microarrays could potentially be used to further define these complex interactions. While the recent advances in MS techniques show great promise for the future, further validation with randomized, prospective trials are needed.

As proteomics continues to develop, genomic strategies have yielded considerable information on lung cancer pathogenesis and potential for therapeutics. Classically, the genetic abnormalities can be through activation of oncogenes (such as K-ras, *myc*, EGFR and *Met*) or inactivation of tumor suppressor genes (such as p53 and Rb). Described below are some relevant genes in lung cancer, and their large potential for therapeutics.

oncogenes

K- Ras

Ras genes are expressed in a number of mammalian cells, and are involved in regulating growth signal transduction. Three forms of Ras have been described in humans: H-Ras, N-Ras and K-Ras. However, the majority of mutations in NSCLC affect the K-Ras gene [18]. K-Ras encodes a group of 21-kDa proteins, the p21 proteins, which are bound to GTP in their active state. Cell proliferation is initiated through Ras-dependent kinases that are inactivated when GTP-ase-activating protein hydrolyzes GTP to guanosine diphosphate. Point mutations have been described at codons 12, 13 and 61, which result in a loss of intrinsic GTP-ase activity. It is estimated that approximately 20% of NSCLC have Ras mutations at codon 12, including 30–50% of adenocarcinomas [19–22]. While an association between smoking and K-Ras mutations has been described, a recent report showed that the incidence of K-Ras mutations in patients with adenocarcinomas who had never smoked was 15%, compared with 25% in patients who were smokers [19,23,24]. In addition, the K-Ras mutation profile in the nonsmokers was distinct from that found in patients who smoked, highlighting the biologic heterogeneity in this particular subset of patients.

The prognostic significance of K-Ras mutations was evaluated in a number of studies with conflicting results. Some studies suggest that activating mutations of K-Ras may represent an adverse prognostic factor. Tumor samples of 69 patients with resected adenocarcinoma were analyzed for K-Ras mutations at codon 12. Those with a mutation present had a significantly higher mortality rate when compared with those who did not (63 vs 32% at 3 years) [22]. In addition, a metaanalysis of 28 trials with a total of 3620 patients found that K-Ras mutations appeared to confer a worse prognosis in patients with NSCLC when detected by PCR, but not by immunohistochemistry (IHC), with a hazard ratio (HR) of 1.35 (95% confidence interval [CI]: 1.16–11.56). This negative prognostic value appeared to apply to patients with adeno carcinoma, with a HR of 1.59 (95% CI: 1.26–2.02), but not to those with squamous cell carcinoma. There were several limitations to this analysis, as all of the trials were retrospective and there was significant variability in the way Ras mutations or p21 overexpression was assessed (i.e., PCR vs IHC) [25]. In other studies, this association is not seen, or is only demonstrated in small subsets of patients [26,27]. In an analysis of 260 patients with resected stage I and II NSCLC, no statistically significant difference in survival was seen between patients with K-Ras mutations and those without mutations. Although, in a subgroup analysis of patients with stage II disease, those with K-Ras mutations had a median survival of 13

months, compared with 38 months for those with wild-type K-Ras [27]. A recent study by Tsao *et al.* also failed to demonstrate a prognostic role for Ras mutations in patients with resected stage IB and II NSCLC [28].

While there are a lack of compelling data to support the use of K-Ras as a prognostic marker in NSCLC, recent data regarding K-Ras mutational status and the response to anti-EGFR therapy in colorectal carcinoma raises the issue of the utility of K-Ras as a predictive marker. In this study, tumor samples were analyzed for K-Ras mutations in 427 patients with chemotherapy-refractory metastatic colorectal carcinoma. While 17% of patients with wild-type K-Ras responded to therapy with panitumumab, no patients with mutant K-Ras had a response [29]. Previous studies have not shown K-Ras mutational status to be predictive of a benefit from standard adjuvant chemotherapy in NSCLC [26,28]. However, with recent data demonstrating the efficacy of the EGFR-targeted monoclonal antibody cetuximab in first-line treatment for advanced NSCLC, identifying those patients who are most likely to benefit from targeted therapy will be critical [30].

c- MET

c-MET is a heterodimeric tyrosine kinase receptor that is expressed in both normal and malignant cells [31–33]. The *MET* gene is located on chromosome 7, and several mutations have been reported in a wide variety of human cancers, including both NSCLC and SCLC. c-MET, via signaling through its natural ligand HGF/scatter factor, is important for numerous cellular processes, including cell proliferation, angiogenesis, invasion and metastasis [34]. Additional downstream effectors of c-MET include PI3K, Ras, p85 and the p21 GTPases. A number of mutations have been reported in a variety of human cancers, with most of the mutations occurring in the tyrosine kinase domain, resulting in constitutive activity [35]. Interestingly, tyrosine kinase domain mutations are rare in lung cancer, whereas mutations in the juxtamembrane domain or semaphorin domain are common in c-MET. It is also thought that HGF, when produced by tumor or mesenchymal cells, may act in an autocrine or paracrine fashion to activate c-MET expressed in epithelial cells [36].

Overexpression of c-MET is thought to confer a poor prognosis in lung cancer. In addition to prognosis, it may also have important implications in the diagnosis and detection of minimal residual disease. In one study, c-MET over expression was found in 24 out of 30 adenocarcinomas and 10 out of 15 squamous cell carcinomas (SCCs). In patients whose tumors were found to overexpress c-MET, 23 (67%) were found to have overexpression of circulating c-MET compared with none of the 11 patients whose tumor did not express c-MET. Results from this study showed that, as a molecular marker for NSCLC, circulating c-MET had a sensitivity and specificity of 51.1 and 96.8%, respectively. Furthermore, there is evidence that overexpression of circulating c-MET mRNA correlated with N-stage as well as early recurrence [37]. In addition, Siegfried *et al.* measured immunoreactive HGF (ir-HGF) in 56 resected NSCLCs, and found that ir-HGF levels higher than the median were associated with decreased survival. Patients with stage I disease who had elevated ir-HGF levels had a worse outcome than patients with stage II or III disease who had low ir-HGF values, with a mortality of 55 versus 15%. While this study was carried out in a small number of patients, it implies that over-expression of HGF, and potentially its receptor, c-MET, is an independent prognostic variable in patients with NSCLC.

EGFR

The family of EGF receptors, including EGFR/HER1, HER2/neu, HER3 and HER4 are members of the erbB gene family, which are involved in a wide variety of cellular processes, including proliferation, suppression of apoptosis, cell motility and angiogenesis. The EGFR, or c-erbB-1, is a transmembrane receptor tyrosine kinase with downstream ligands that include

EGF, TGF-α, β-cellulin and epiregulin. EGFR has been reported to be overexpressed in a number of human malignancies, including 43–89% of NSCLCs, depending on the case series [38]. It has also been found to be overexpressed in pre-malignant bronchial lesions, thereby implying a critical role in tumorigenesis [39,40]. A number of groups have described EGFR mutations in the tyrosine kinase domain; approximately 90% are caused by either short, in-frame deletions in exon 19 or a point mutation at amino acid 858 that results in the substitution of arginine in place of leucine (L858R). It has been established that overexpression is most common in SCC, followed by adenocarcinoma and bronchioalveolar carcinoma. Furthermore, it has been shown that EGFR is more likely to be overexpressed in well-differentiated histology than in poorly differentiated histology.

The prognostic significance of aberrations in the EGFR pathway in lung cancer remain controversial. A number of methodologies have been used to assess this gene, including immmunohistochemistry, screening for gene mutations, and analysis of gene copy number. Several studies have shown that EGFR overexpression, as determined by immunohistochemistry, appears to confer a poor prognosis in patients with NSCLC [41–44]. However, these results have not been confirmed in other studies [45–47]. Hirsch *et al.* analyzed tissue from 183 patients with NSCLC. EGFR overexpression was assessed by immunohistochemistry and EGFR gene copy number was analyzed by FISH. Although a high gene copy number per cell showed a trend towards a worse prognosis, this was not statistically significant [38]. Given the conflicting data that are currently available, EGFR does not have a clear role as a prognostic marker in NSCLCs.

With the recent advances in the development of EGFR-targeted therapy, however, it has become increasingly important to define predictive markers that identify a subset of patients who are most likely to benefit from EGFR-TKI therapy. In four randomized trials, the addition of gefitinib or erlotinib did not confer a survival advantage when added to a platinum doublet as first-line treatment in patients with NSCLC [48–51]. Subsequent trials of EGFR-TKIs in patients who have been previously treated have shown an improvement in both survival and quality of life when compared with placebo groups [52,53]. From analysis of the literature, it is apparent that certain clinical and pathologic characteristics are predictive of a better response to EGFR-TKIs, including patients who are of Asian origin, female, never smoked and those who have adenocarcinomas [54]. In addition, a strong positive correlation has been seen between cutaneous toxicity and response rate to treatment in a number of studies [55,56]. The role of EGFR as a molecular predictive marker for response to therapy is less clear. Retrospective studies have suggested that there is an association between EGFR mutations and response to EGFR-TKIs that ultimately translates into an improvement in overall survival [57]. However, in larger, randomized trials, this was not confirmed. In the BR.21 trial, while EGFR mutations were found to be predictive of a better response to erlotinib, no survival advantage was seen [58]. Additional studies have focused on EGFR gene copy number as a means of identifying those patients who are most likely to benefit from targeted therapy. Several studies have reported that in patients who are treated with EGFR-TKIs, those whose tumors are FISH-positive have significantly higher response and survival rates when compared with patients whose tumors are FISH-negative [58–60]. However, it should be noted that FISH analysis failed to identify a subset of patients who benefited from gefitinib when compared with docetaxel in the second-line setting for advanced NSCLCs [55].

Resistance to EGFR-TKIs

Resistance to EGFR-targeted therapy is a complex process, and sensitivity to EGFR-TKIs may be dependent on the specific mutation present [61]. Resistance is often caused by additional acquired mutations; one of the most extensively studied being T790M, which alters the conformation of the tyrosine kinase domain, preventing binding of erlotinib and gefitinib

[62,63]. Recent data suggest that *MET* amplification represents an additional mechanism that leads to gefitinib resistance through EGFR-independent activation of the PI3K pathway [64]. COX-2 over expression has also been implicated as a cause of EGFR-TKI resistance. In NSCLC cells, it was found that the COX-2 product, prostaglandin E2, activated the mitogen-activated protein kinase/Erk pathway independently of EGFR [65]. While preclinical data suggest that there may be some benefit to combined inhibition, a study of gefitinib and celecoxib as first-line treatment in advanced NSCLC had a lower response rate compared with that expected for standard combination chemotherapy [66].

Novel EGFR-targeted agents have been designed in an attempt to overcome resistance [60]. One of the most common truncated receptor mutations seen is the EGFR variant III, which results from an in-frame deletion of exons 2–7. This mutation was initially described in glioblastoma, and is present in approximately 5% of SCC of the lung. It has been demonstrated in a murine model that the administration of the irreversible EGFR inhibitor, HKI-272, results in more a more dramatic shrinkage of EGFR variant III-driven tumors when compared with erlotinib [67]. In addition, in preclinical studies, other irreversible EGFR-TKIs, such as XL647, show inhibition of erlotinib/gefitinib-resistant mutants [68]. Another second-generation TKI, BIBW 2992, has demonstrated tolerability in phase I studies [69].

Tumor suppressor genes

p53

The p53 gene is located on chromosome 17, and encodes for a 53-kD nuclear phospho protein, which acts as a transcription factor that blocks cell cycle progression from the G1 phase. It plays a critical role in the regulation of cell cycle events, specifically in response to DNA-damaging agents. Mutations in this gene result in loss of inhibition of proliferation, and have been associated with lung cancer more than any other genetic abnormality. Mutations in p53 have been reported in 20–60% of NSCLCs as well as in SCLCs. In an analysis of 820 heavy smokers without cancer, p53 mutations were identified in the sputum of approximately 2% of the patients, potentially signifying those who are at a higher risk of developing lung cancer [70]. In addition, a meta-analysis in patients with resected NSCLC found that p53 abnormalities occurred more frequently in patients with histologies that were more strongly associated with smoking, that is, squamous cell and large cell carcinoma versus adenocarcinoma [71]

While p53 mutations have been studied extensively in lung cancer, their prognostic significance remains controversial. Two meta-analyses report on the impact of p53 mutations or overexpression on prognosis. An abnormal p53 status, as detected by a number of methods, including IHC, PCR and ELISA, was found to have a combined HR of 1.44 (CI: 1.2–1.72), suggesting an unfavorable impact on survival in all types of lung cancer, regardless of stage [72]. There were insufficient data to report on SCLC. The second meta-analysis found that p53 alterations conferred a poorer prognosis in patients with adenocarcinoma, but had no impact on survival in patients with SCC [73]. These studies have been limited by their retrospective nature and the insensitivity of the p53 detection techniques. In a prospective trial of 188 patients with resectable lung cancer, tumor samples were analyzed by direct gene sequencing. p53 was found to be mutated in 55% of tumors; however, it only appeared to be statistically significant in patients with stage I NSCLC, and a HR for death of 2.8 (CI: 1.4–5.6). In a subset analysis of these patients, those with mutations in p53 that would be expected to alter the structure or function of the gene had a significantly reduced 4-year survival rate than patients with wild-type p53 (37 vs 62%, respectively). Patients with other mutations in p53 did not have statistically different overall survival when compared with those with wild-type p53 [74]. With improved molecular techniques, it is now possible to assess the specific gene mutation, thereby allowing the identification of a subset of patients who may benefit from additional therapy with either standard or novel agents.

There is growing evidence that p53 may have clinical utility as a predictive marker. Shih *et al.* reported that the level of p53 expression detected by RT-PCR in the peripheral blood of patients was significantly elevated in patients whose disease was resistant to cisplatin-based chemotherapy [75]. Tumor samples from a large phase III trial in which patients with stage IB or II NSCLC were randomized to receive either cisplatin plus vinorelbine or observation, were analyzed for both p53 overexpression and mutations. Patients who overexpressed p53 had a significantly shorter overall survival, with a HR of 1.89 (CI: 1.07–3.34). Interestingly, these patients appeared to derive a greater survival benefit from chemotherapy, with a HR of 0.54 (CI: 0.32–30.92). p53 mutational status did not appear to have any prognostic or predictive significance [28]. While these studies highlight a potential subset of early-stage patients who may benefit from aggressive adjuvant therapy, the use of p53 as a predictive tool remains unclear, and additional prospective studies are warranted.

Rb

Although originally described in retinoblastoma, the retinoblastoma (*Rb*) gene has been shown to be implicated in the carcinogenesis in a large number of solid tumors. The *Rb* gene is located in chromosome 13, and encodes for a nuclear protein that functions as a tumor suppressor. This gene plays a critical role in cell cycle regulation during the G0–S phase, and phosphorylation through the action of CDK4–cyclin D and CDK2–cyclin E complexes causes the gene to become inactivated, thereby allowing cell cycle progression. Mutations in the *Rb* gene have been reported in over 90% of SCLCs and approximately 15% of NSCLCs [76].

Loss-of-function of *Rb* has been associated with a poorer prognosis in patients with NSCLC, and occurs through a variety of mechanisms, including mutation and dysregulated phosphorylation [77]. There is also evidence that the effects of the loss of function of the *Rb* gene may be synergistic when occuring with other molecular abnormalities. Specimens from 119 patients with stage I and II adenocarcinomas were analyzed for Rb expression by IHC. Patients whose tumors expressed a mutant p53, but did not express Rb had a significantly shorter overall survival when compared with patients who expressed Rb [78]. While studies have demonstrated a correlation between the absence of the Rb protein and poor prognosis [79], in some studies this association is not seen [80,81], and the significance of Rb as a prognostic marker in NSCLC remains controversial. There is also some evidence that NSCLC xenograft models with a deficiency in Rb demonstrate more aggressive behavior, yet are more sensitive to chemotherapeutic agents, as the normal cell cycle arrest mechanism is no longer intact [82].

DNA repair mechanisms

While a number of molecular and genetic markers that have prognostic significance in patients with lung cancer have been identified, efforts have also focused on identifying molecular markers that predict response to treatment. A significant percentage of patients with NSCLC, who are initially deemed surgically resectable, ultimately recur and die of their disease. Although the benefit of platinum-based chemotherapy in the adjuvant setting has been demonstrated [83], its effect is modest at best. It is clear that a significant proportion of patients are being treated and exposed to the risks associated with chemotherapy and do not benefit from treatment. DNA repair mechanisms, specifically nucleotide excision repair, are critical in the development of resistance to platinum chemo-therapeutic agents. Effectors in this pathway could serve as potential markers that could reliably predict recurrence risk after surgery as well as response to platinum-based chemotherapy in the adjuvant setting.

ERCC1/RRM1

The excision repair cross complementation group 1 (ERCC1), the regulatory subunit of ribonucleotide reductase (RRM1) are part of the nucleotide excision repair pathway (NER), and play a critical role in tumorigenesis and cancer progression. The NER pathway is one of the most important DNA repair pathways, and is responsible for the repair of a variety of DNA-distorting lesions, including those caused by cisplatin adducts and tobacco carcinogens. The genetic mutations that were initially described in xeroderma pigmentosum (XP), in which patients are extremely susceptible to UV light-induced skin damage, are now known to represent seven key genes in the NER pathway (XP A–G). The pathway functions by initially recognizing the area of damaged DNA, followed by a localized opening of the helix at the site of the damage. A variety of factors are then recruited that result in DNA excision and gap filling. ERCC1, along with its cofactor XPF, is involved in these later steps, where it is responsible for creating a nick at the 5′ end of the damaged DNA [84].

The components of this pathway also appear to have an effect on the response of NSCLC to both surgical treatment and chemo therapy. Low levels of RRM1 have been associated with decreased survival in patients with NSCLC [85]. *In vitro*, expression of RRM1 has been shown to increase the expression of phosphatase and tensin homologue, which inhibits cellular proliferation. In a study of 187 patients with early-stage NSCLC who were surgically resected and did not receive adjuvant chemotherapy, increased levels of RRM1 conferred a favorable prognosis. In patients who had increased RRM1 expression, the median overall survival exceeded 120 months, as compared with a median survival of 60.2 months for those with low levels of RRM1. In this particular analysis, RRM1 was the only variable associated with disease-free survival and appeared to correlate with ERCC1 expression [86].

The role of ERCC1 and RRM1 levels in predicting the response in patients receiving chemotherapy has also been investigated. The International Adjuvant Lung Cancer Trial (IALT) has demonstrated that adjuvant cisplatin-based chemotherapy improves survival in patients with resected NSCLC, but this effect is modest, with an absolute improvement in 5-year overall survival of only 4–15%. ERCC1 plays an important role in DNA repair, and recognizes DNA–platinum adducts. In analyzing 761 tumor samples of patients who had enrolled on the IALT study, it was found that patients with ERCC1-negative tumors appeared to benefit from cisplatin-based chemotherapy, with a significantly prolonged survival when compared with those with ERCC1-positive tumors. In patients who did not receive chemotherapy, those with ERCC1-positive tumors had a longer survival, with HR for death of 0.66 (CI: 0.49–0.9) [87]. In attempting to account for the conflicting results that ERCC1 and RRM1 expression have on prognosis, it has been postulated that these DNA-repair mechanisms play different roles in early-versus later-stage disease. It is thought that early in the course of lung cancer, it may be beneficial to have high levels of ERCC1 and RRM1 to prevent additional DNA mutations that may lead to increased cellular proliferation and invasiveness. However, these same mechanisms may, ultimately, lead to drug resistance in patients treated with cytotoxic chemotherapy. Cobo *et al.* performed RT-PCR to assess ERCC1 mRNA expression in tissue samples of patients with stage IV NSCLC. Patients who were assigned to the genotypic arm received docetaxel plus cisplatin if they had low ERCC1 levels, while those with high levels received docetaxel and gemcitabine. Objective response was significantly higher in the genotypic arm at 50.7%, compared with the control arm, which had a response rate of 39.3% [88]. Thus, while there is promise that these molecular markers may aid in tailoring therapeutic decisions, additional prospective trials are warranted.

Conclusions

While progress has been made in recent years, the overall prognosis for patients diagnosed with lung cancer remains poor, with mortality from lung cancer exceeding that from breast,

colon and prostate cancers combined. Traditional prognostic markers such as age, stage and performance status will continue to have a role in the management of patients with this disease. It is clear, however, that lung cancer is a hetero geneous disease, and that more sophisticated ways of risk-stratifying patients and tailoring therapies are needed. Biomarkers have the potential to afford earlier disease detection, as well as assigning prognosis and guiding treatment decisions. While it is unlikely that a single marker will fulfill all of these criteria, an increased understanding of the multiple pathways involved in lung carcinogenesis may lead to improved outcomes in the future.

Future perspective

It is clear that lung cancer is a complex and heterogenous disease. While a number of promising molecular markers have been identified, no one marker exists that can reliably predict prognosis or response to treatment for patients affected by this devastating disease. As the molecular biology of lung cancer becomes more clearly defined, the development of more targeted therapeutics will be possible. Traditional prognostic markers, such as age, stage and performance status will still undoubtedly be valuable; however, the identification of novel markers may help to further refine this and guide treatment decisions. The fields of proteomics and genomics hold great promise for identifying potential therapeutic targets as well as predicting response to treatment.

Over the next several years, it may indeed be possible to identify patients who will benefit from treatment based on their molecular or genomic profile, and to tailor chemotherapeutic regimens accordingly.

Executive summary

- Serum markers have been studied extensively in lung cancer, although no marker has been identified as a reliable predictive or prognostic marker.
- The field of proteomics offers new promise in attempting to identify novel biomarkers.
- K-Ras is mutated in approximately 20% of non-small-cell lung cancer (NSCLC) cases and remains an attractive target for monitoring disease status, although additional prospective trials are warranted. K-Ras may represent an important predictive marker for response to EGF receptor (EGFR)-targeted therapy in NSCLC.
- c-MET mutations are found in both NSCLC and small-cell lung cancer (SCLC), and are thought to confer a poor prognosis.
- EGFR has been reported to be overexpressed in 40–90% of NSCLC cases. The prognostic significance of EGFR overexpression remains controversial; however, with the development of EGFR-targeted therapy, it is now critical to identify subsets of patients who are most likely to benefit from therapy.
- p53 mutations have been reported in 20–60% of NSCLCs, as well as SCLCs. Conflicting data have been reported regarding the predictive and prognostic value of this marker, although advances in molecular techniques may identify specific mutations that are clinically significant.
- Rb mutations have been reported in over 90% of SCLC and 15% of NSCLC cases. Loss-of-function of *Rb* may confer a worse prognosis in patients with NSCLC.

- ERCC1 and RRM1 are critical components of the nucleotide excision repair pathway. Patients with ERCC1-negative tumors appear to benefit from cisplatin-based chemotherapy. In patients who do not receive chemotherapy, those with ERCC1-positive tumors survive significantly longer.

Acknowledgments

Financial & competing interests disclosure

Supported in part by NIH/NCI, MARF (Jefferey P Hayes Memorial), Cancer Research Foundation and V-Foundation (R Salgia). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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