



## Lung cancer • 9: Molecular biology of lung cancer: clinical implications

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## REVIEW SERIES

## Lung cancer · 9: Molecular biology of lung cancer: clinical implications

K M Fong, Y Sekido, A F Gazdar, J D Minna

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It has been hypothesised that clinically evident lung cancers have accumulated many different genetic or epigenetic abnormalities in oncogenes and/or tumour suppressor genes. This notion has important clinical ramifications. Recent developments in our knowledge of the molecular biology of lung cancer are reviewed, with particular reference to genetic abnormalities in tumour suppressor gene inactivation and overactivity of growth promoting oncogenes. These changes lead to the "hallmarks of lung cancer". These hallmarks are the new rational targets for early detection, prevention, and treatment of lung cancer.

than in the small number of preneoplastic cells in smoking damaged respiratory epithelium which require special study methods. Nonetheless, such techniques as precise laser capture microdissection now allow the molecular testing of minute specimens and can reveal DNA lesions as well as aberrant gene expression to complement traditional methods such as immunohistochemistry. At the other end of the spectrum, gene microarrays are making it possible to study the expression of thousands of genes in individual lung cancers, with initial studies showing that it is possible to reproducibly classify lung cancers into relevant histological and clinical prognostic groups based on their gene expression profiles.<sup>7-8</sup> Such advances bode well for learning more about the biology of lung cancers and, to the clinician, the potential also to provide novel predictive information on tumour behaviour, survival, and response to treatment.

The advances in molecular technologies are providing insight into the pathobiology of lung cancer development. It is becoming apparent through candidate gene and genome wide approaches that clinically evident lung cancers have accumulated numerous (perhaps 20 or more) clonal genetic and epigenetic alterations as a multistep process. These alterations include the classical genetic abnormalities of tumour suppressor gene (TSG) inactivation and overactivity of growth promoting oncogenes. More recently, tumour acquired promoter hypermethylation has been recognised as a mechanism for the epigenetic inactivation of gene expression. The early clonal genetic lesions that occur in smoking damaged preneoplastic bronchial epithelium are being identified, as are the molecular differences between small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), and between tumours with different clinical outcomes. These abnormalities lead to the "hallmarks of lung cancer". Molecular studies are now performed in many research laboratories in an integrated approach with clinical investigators. These will lead to clinical applications with the potential to provide new avenues for early diagnosis, risk assessment, prevention, and treatment for this common and highly lethal condition.<sup>1-2</sup>

Smoking is the major cause of most cases of lung cancer and is the result of nicotine addiction and cigarette smoke carcinogens (reviewed in Hecht<sup>9</sup>). Our major efforts must therefore continue to be preventing smoking initiation and helping with smoking cessation through a variety of methods.<sup>10</sup> Nevertheless, recent molecular studies show that genetic damage to the smoking exposed respiratory epithelium persists for decades after smoking cessation, and in the USA about 50% of all lung cancers currently occur in former cigarette smokers; we will therefore need to deal with the possibility that lung cancer can develop even after smoking cessation.<sup>9-11</sup> It is likely that lung cancer cells start to harbour genetic damage after prolonged exposure to tobacco smoke, with changes demonstrable in morphologically normal cells from smokers.<sup>11-14</sup> It appears that genetic damage continues to accumulate in bronchial epithelial cells in line with traditional indices of increasing preneoplastic morphology. Ultimately, multiple clonal lesions are detectable in overt invasive lung cancers, and perhaps even more in metastatic lesions, consistent with the multistep model of carcinogenesis. The genetic and cellular targets of the carcinogenic process are notably diverse, yielding the following "hallmarks of cancer"<sup>15</sup>:

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#### SPECTRUM OF MOLECULAR ALTERATIONS RESULTS IN THE HALLMARKS OF LUNG CANCER

Recent molecular developments are increasing our knowledge of the changes somatically acquired by lung cancer cells during their pathogenesis.<sup>3-6</sup> Much more is known about the molecular abnormalities that occur in clinically overt lung cancers, which are more easily studied

- abnormalities in self-sufficiency of growth signals;
- evading apoptosis;
- insensitivity to anti-growth signals;
- limitless replicative potential;
- sustained angiogenesis; and

- tissue invasion and metastases.

Abnormalities in DNA repair and a genetic predisposition to develop lung cancer, particularly with exposure to smoking, sets the stage for the development of this carcinogenic process.

### Self-sufficiency of growth signals: proto-oncogenes and growth stimulation by autocrine and paracrine factors

A number of growth factors and their cognate receptors are expressed by lung cancers or their adjacent stromal cells, thus producing autocrine and paracrine growth stimulation loops. Several are encoded for by proto-oncogenes which become activated in the course of lung cancer development.<sup>4–6</sup> The ERBB family is a group of transmembrane receptor tyrosine kinases which, together with their ligands, constitutes a potential growth stimulatory loop, particularly for NSCLCs. The two members important for lung cancer are the epidermal growth factor receptor (EGFR, ERBB1) and HER2/neu (ERBB2), which are expressed independently of one another in NSCLC.<sup>16</sup> On ligand binding, ERBB receptors homodimerise or heterodimerise, thereby inducing intrinsic kinase activities that initiate intracellular signal transduction cascades including the MAP kinases. EGFR regulates epithelial proliferation and differentiation and can be over-expressed in lung cancers. Moreover, lung cancer cells also express ligands for EGFR such as epidermal growth factor (EGF) and transforming growth factor  $\alpha$  (TGF $\alpha$ ), thereby producing a potential autocrine growth loop.<sup>17–19</sup> Some, but not all, studies have associated EGFR expression with impaired survival.<sup>16 20–24</sup> Monoclonal antibodies against the EGFR (C225, ImClone) are entering clinical trials in combination with chemotherapy.<sup>25</sup> In addition, tyrosine kinase inhibitors that have some selectivity such as ERBB1 blockers (CP358774, ZD1839-Iressa, OSI774) are also being tested, most with the advantage of being orally active. Another ERBB family member, HER2/neu, is highly expressed in about 30% of NSCLCs, especially adenocarcinomas.<sup>18 26</sup> High HER2/neu levels are associated with the multiple drug resistance phenotype<sup>27</sup> and increased metastatic potential in NSCLC,<sup>28</sup> which may help to explain the poor clinical outcome linked to HER2/neu overexpression reported by some but not all investigators.<sup>16 22 24 29</sup> Clinical trials investigating chemotherapy combined with trastuzumab (Herceptin), a monoclonal antibody against the HER2/neu receptor, are in progress in lung cancer.<sup>30</sup>

The autocrine loop comprising stem cell factor and its tyrosine kinase receptor CD117 is activated in some lung cancers—more often in SCLC than NSCLC—with resultant growth promotion or chemoattraction. The recent development of specific tyrosine kinase inhibitors to target this pathway may translate into novel approaches for this highly lethal subtype.<sup>31–33</sup> Similarly, the gastrin releasing peptide (GRP) growth stimulatory loop is involved in 20–60% of SCLCs.<sup>34</sup> The therapeutic potential of inhibiting this pathway with a neutralising monoclonal antibody directed against GRP, as well as by antagonists of GRP (also referred to as bombesin), is being tested in early clinical trials of SCLC.<sup>35 36</sup> The GRP receptors belong to a G-protein coupled receptor superfamily including GRP-, neuromedin B- and bombesin subtype-3 receptors; all of these can be expressed in lung cancers of all histological types and some bronchial epithelial biopsies from smokers, implying an early pathogenic role for this family.<sup>37 38</sup> The GRP receptor is expressed more frequently in women (where there are two expressed copies of the X linked gene) than in men in the absence of smoking. Its expression is activated earlier in women in response to tobacco exposure, which may be a factor in the increased

susceptibility of women to tobacco induced lung cancer.<sup>39</sup> Other putative growth factor systems include insulin-like growth factors (IGF) I and II, the type I IGF receptor, platelet derived growth factor/receptor, and the hepatocyte growth factor/receptor.<sup>5 40</sup> Each of these should be further studied for any potential clinical usefulness. Insulin-like growth factor binding protein-6 (IGFBP-6) activated programmed cell death in NSCLC cells while IGFBP-3 inhibited cell growth in human lung cancers, suggesting that these binding proteins might potentially be new treatments.<sup>41 42</sup> In addition, high levels of blood IGF-I and enhanced mutagen sensitivity of peripheral blood lymphocytes were individually associated with an increased risk of lung cancer, which suggests that genetic polymorphisms in IGFs may predispose to the development of lung cancer.<sup>43</sup>

The RAS proto-oncogene family (KRAS, HRAS, and NRAS) which encodes 21 kD plasma membrane proteins comprises an important signal transduction pathway. Its members, especially KRAS, can be activated in some lung cancers by point mutations, leading to inappropriate signalling for cell proliferation. The KRAS gene is frequently mutated at codon 12 and also at codons 13 and 61. Mutations are found in 15–20% of all NSCLCs apart from SCLCs, especially adenocarcinomas (20–30%).<sup>34</sup> KRAS mutations correlate with smoking,<sup>44</sup> often being the G–T transversions associated with polycyclic hydrocarbons and nitrosamines.<sup>45</sup> In mice, somatic activation of KRAS by spontaneous recombination predisposes the animals to tumours, predominantly early onset lung cancer.<sup>46</sup> While the prognostic importance of KRAS mutations is debated,<sup>47–51</sup> it does not appear to predict the response to chemotherapy.<sup>52</sup> Two recent large studies in resected NSCLC showed that KRAS mutations were independent but weak predictors of survival.<sup>53 54</sup> The necessity for the Ras protein to undergo farnesylation to become active has led to the development of specific inhibitors of the responsible farnesyltransferase enzyme. Several of these agents are currently in clinical trials against lung cancer (for example, BMS214662, RII5777, SCH 66336), and trials of vaccination with mutant KRAS peptides are also underway.<sup>55</sup> Other potential avenues to block RAS include antisense treatment, inhibition of protein expression, or downstream effectors.<sup>56</sup>

The MYC proto-oncogene family encodes nuclear products which are the ultimate target of RAS signal transduction; the most frequently involved family member is c-MYC in both SCLC and NSCLC, unlike MYCN and MYCL which are generally activated only in SCLC. Activation occurs as a result of protein overexpression caused by gene amplification or by transcriptional dysregulation. There also appears to be a change in lung cancers leading to increased stability of MYC mRNA.<sup>57</sup> Approximately 18–31% of SCLCs had amplification of one MYC family member compared with 8–20% of NSCLCs.<sup>34</sup> MYC amplification appears to occur more frequently in chemotherapy treated patients, and the “variant” SCLC subtype may correlate with adverse survival. Recent studies have suggested that low levels of MYC amplification occur in NSCLC and are associated with impaired survival; the combination of MYC expression with loss of caspase-3 (an apoptosis inducer) expression results in worse survival.<sup>58 59</sup> MYC expression may represent an avenue for therapeutic manipulation. For instance, the growth inhibition of an SCLC cell line by all-trans-retinoic acid appears to be associated with increased MYCL and decreased MYC expression.<sup>60</sup> Moreover, antisense therapy strategies directed at downregulating MYC expression appear encouraging in cell culture systems.

### Evading apoptosis

Tumour cells often escape the normal physiological response (termed programmed cell death or apoptosis) when challenged

by cellular and DNA damage. Key players include the p53 gene and the *BCL2* proto-oncogene. *BCL2* protects against apoptosis and its expression is higher in SCLC (75–95%) than in NSCLC. These findings are seemingly unexpected as SCLCs are more sensitive to chemotherapy, which often induces an apoptotic response. In any case, the prognostic value of *BCL2* expression is controversial.<sup>5 61</sup> *BCL2* expression in tumours actually predicts increased survival of patients with NSCLC.<sup>62</sup> *BAX* is a *BCL2* related protein which promotes apoptosis and is a downstream transcription target of p53. *BAX* and *BCL2* expression is inversely related in neuroendocrine cancers; high *BCL2* and low *BAX* expression occurs in most SCLCs which are usually p53 deficient.<sup>63</sup> Expression of the inhibitor of apoptosis protein (IAP)-1 acts as an important anti-apoptotic protein mediating sensitivity to deoxynucleotide analogues in NSCLC cells.<sup>64</sup> Among the anti-apoptosis strategies in preclinical trials are studies of antisense *BCL2* in SCLC (to downregulate *BCL2* protein expression), *BCL-xL* antisense in NSCLC, and a bispecific *BCL2-BCLxL* antisense to target both SCLC and NSCLC.

### Insensitivity to anti-growth signals: tumour suppressor genes (TSGs)

TSGs play a critical role in controlling normal cell growth. They generally inhibit the tumorigenic process but can also be involved in the response and repair of DNA damage. TSGs are rendered inactive by chromosomal loss of one allele (loss of heterozygosity (LOH)) and damage to the other by genetic mutation or the epigenetic hypermethylation of its promoter. Studies of LOH as a marker of TSG inactivation have shown that a number of chromosomal regions are damaged in overt lung cancer cells. For instance, a genome wide search for LOH in 36 lung cancer cell lines using ~400 high resolution polymorphic markers showed that tumours had a mean of 17–22 “hot spots” of chromosomal loss.<sup>65</sup> There were 22 different regions with more than 60% LOH, 13 with a preference for SCLC, seven for NSCLC, and two affecting both histological types. The sharing of some LOH regions and the specificity of others may provide an insight into the genes common to lung cancer development and others specific to subtype differentiation. The chromosomal arms with the most frequent LOH were 1p, 3p, 4p, 4q, 5q, 8p, 9p (p16 TSG locus), 9q, 10p, 10q, 13q (RB-retinoblastoma TSG locus), 15q, 17p (p53 TSG locus), 18q, 19p, Xp, and Xq.

There is an intense hunt for the candidate genes in chromosomal regions with high frequencies of LOH where the precise TSG is not known. For example, several candidate genes are located on 3p where LOH can be found in up to 96% of lung cancers and 78% of preneoplastic/preinvasive lesions,<sup>66</sup> as well as by homozygous deletions.<sup>3</sup> The frequency and size of 3p LOH increased with the severity of histopathological preneoplastic/preinvasive changes. One candidate TSG is *FHIT* at the 3p14.2 region; lung cancer cells frequently express abnormal mRNA transcripts of *FHIT* apart from normal wild type transcripts.<sup>67 68</sup> Re-introduction of exogenous *FHIT* may suppress the tumorigenicity of a lung cancer cell line in nude mice.<sup>69 70</sup> There are also TSG candidates at the 3p21.3 region which appear to suppress the tumorigenic phenotype when introduced back into lung cancers with numerous other genetic lesions.<sup>71</sup> One such is the *RASSF1A* mRNA isoform at the *RASSF1* locus<sup>72–74</sup>; while the gene is rarely mutated, its expression is lost by promoter acquired hypermethylation in ~90% of SCLCs and 30–40% of NSCLCs. Methylation in NSCLCs is associated with adverse survival, and treatment of lung cancer cells with 5-aza-2′ deoxycytidine reactivates *RASSF1A* expression. *RASSF1A* acts to inhibit DNA synthesis and downregulate the expression of cyclin D1 at a post-transcriptional level. Also located in the 3p21 region with 5 kb and 50 kb, respectively, are the

*FUS1* and *SEMA3B* genes.<sup>71 75 76</sup> Expression of wild type but not tumour acquired mutant *FUS1* dramatically suppresses the growth in vitro of lung cancer cells, while systemic delivery of *FUS1* in an adenovirus vector resulted in regression of metastatic disease in a lung cancer mouse xenograft model.<sup>76</sup> Wild type *SEMA3B* reintroduced into lung cancer cells induces apoptosis, unlike *SEMA3B* missense mutants.<sup>77</sup> In addition, transfection of *SEMA3B* into cells results in conditioned media that induce the death of lung cancer cells, which raises the possibility of using this soluble secreted protein as a systemic anticancer treatment.<sup>77</sup> Other 3p genes with evidence of tumour suppressor activity are *DUTTI/ROBO1*, which was associated with inadequate lung development and bronchial hyperplasia in mice with a targeted deletion of the gene,<sup>78</sup> and retinoic acid receptor (*RAR*)  $\beta$  which frequently undergoes LOH and promoter hypermethylation.<sup>79</sup> There are therefore a number of candidate 3p TSGs, and LOH here may possibly be the earliest acquired genetic change in lung cancer development

Other chromosomal regions affected by LOH in lung cancers house known TSGs such as p53, retinoblastoma (RB), and p16, and these are often found to be abnormal by immunohistochemical examination in lung cancer.<sup>80</sup> p53 is a key TSG; its protein helps maintain genomic integrity in the face of DNA damage from  $\gamma$  or UV irradiation and carcinogens. DNA damage or hypoxia upregulates p53 which acts as a transcription factor regulating a number of downstream genes including p21, MDM2, GADD45, and BAX, thereby helping to regulate the G1/S cell cycle transition, G2/M DNA damage check point, and apoptosis. p53 inactivation occurs in  $\geq 75\%$  of SCLCs and about 50% of NSCLCs,<sup>45 53 54 81–83</sup> with mutations correlating with cigarette smoking and comprising the G–T transversions expected of tobacco smoke carcinogens. Missense p53 mutations can prolong the protein half life leading to easily detected mutant p53 protein by immunohistochemistry.<sup>53 54 83</sup> p53 mutations have been linked to response to cis-platinum based chemotherapy in NSCLC<sup>84</sup> and the response to radiotherapy.<sup>85</sup> While there is debate on the prognostic role of p53 abnormalities in NSCLC, the preponderance of evidence suggests that the presence of such abnormalities leads to a worse prognosis.<sup>53 54 83 86</sup> p53 is a prototypic model for gene replacement therapy in lung cancer. Preclinical studies showed that restoring p53 function resulted in apoptosis of cancer cells, and have progressed to phase II clinical trials where adenoviral mediated p53 gene transfer delivered by direct tumour injection appeared feasible when given in conjunction with radiation therapy.<sup>87</sup> Conversely, intratumoral injection of adenoviral p53 appeared to provide no additional benefit in patients receiving first line chemotherapy for advanced NSCLC.<sup>88</sup> Vaccine trials with mutant p53 peptides are also being performed. p53 is kept at virtually undetectable levels in normal cells by an autoregulatory loop involving the production of HDM2, the human homologue of the murine double minute 2 (MDM2) oncogene which blocks p53 regulation of target genes and enhances its proteasome dependent degradation. Conversely, p53 regulates (increases) the expression of HDM2 by directly binding and activating the HDM2 promoter, thereby downregulating itself. The HDM2 protein is overexpressed in 25% of NSCLCs,<sup>89</sup> thus representing another way of abrogating p53 function. HDM2, in turn, is inactivated by p14ARF, the alternative product of the p16 gene whose downregulation is similarly associated with loss of p53/HDM2/p14ARF pathway function.

p16 is part of the p16-cyclin D1-CDK4-RB pathway that is central to controlling the G1–S transition of the cell cycle. This critical cell cycle regulatory pathway is functionally altered or mutated in many cancers including those of lung origin. Each member of the pathway may be rendered

dysfunctional during carcinogenesis. Functional loss of the RB gene can include deletions, nonsense mutations, or splicing abnormalities leading to protein abnormalities in most SCLCs and 15–30% of NSCLCs.<sup>80–90–92</sup> Functionally, in vitro re-introduction into tumour cells of a wild type RB suppresses SCLC growth.<sup>93</sup> Whereas in SCLC the pathway is usually disrupted by RB gene inactivation, cyclin D1, CDK4 and especially p16 abnormalities are common in NSCLC. Cyclin D1 inhibits the activity of RB by stimulating its phosphorylation by cyclin dependent kinase 4 (CDK4). Thus, cyclin D1 overexpression is an alternative mechanism for abrogating this pathway and is found in 25–47% of NSCLC, possibly with a role as a predictor of poor prognosis.<sup>94–95</sup> Furthermore, transfection of a cyclin D1 antisense construct into lung cancer cell lines can be shown to destabilise RB and retard growth.<sup>96</sup> CDK4 expression has also been reported in NSCLCs<sup>97</sup> and an example of potential therapeutic manipulation is flavopiridol. This compound, which inhibits cyclin dependent kinase, is being tested in clinical trials.<sup>98</sup> p16 regulates RB function by inhibiting CDK4 and CDK6 kinase activity. p16 (or CDKN2) is situated on the short arm of chromosome 9 at region 21 and undergoes heterozygous and homozygous loss, mutation, and aberrant promoter hypermethylation in lung cancer, ultimately inactivating its function.<sup>3</sup> Perhaps 30–50% of early stage primary NSCLCs do not express p16. The p16 locus also encodes a second alternative reading frame protein, p14ARF, which functions in the p53/HDM2/p14ARF pathway as discussed above. Interestingly, as an example of their evolutionary deviousness, lung tumours have developed distinct ways of interfering with the two different products from a single genetic locus, each of which functions in a distinct growth regulatory pathway. Moreover, the specific mutational targets differ according to lung cancer subtype, indicating the need for efforts to better understand their relative contribution to tumour differentiation.<sup>61</sup>

#### Tumour acquired promoter hypermethylation as a method of inactivating the expression of TSGs

Tumour acquired promoter methylation is increasingly recognised as an important epigenetic mechanism for inactivating genes. We and others have found tumour acquired aberrant promoter methylation in a number of genes including *RARβ*, *TIMP-3*, *p16*, *MGMT*, *FHIT*, *DAPK*, *ECAD*, *p14ARF*, and *GSTP1*.<sup>79–99–101</sup> At least one gene was methylated in most resected primary NSCLCs, while normal control lung tissue from the same patients was only rarely methylated. About 13% of the NSCLCs exhibited more frequent promoter hypermethylation, suggesting a “global CpG island methylator phenotype”. In NSCLCs hypermethylation contributes to downregulation of p16 expression; it occurs at an early stage in lung carcinogenesis<sup>102–104</sup> and correlates with smoking.<sup>105</sup> Other regional sites of hypermethylation have been found in lung cancer, including sites at 3p (*RARβ*, *FHIT*, *RASSF1A*, *SEMA3B*), 4q34, 10q26, and 17p13, although the precise gene targets at several of these sites are uncertain and the significance is not yet apparent.<sup>106–107</sup> Hypermethylation of certain gene promoters may also have relevance in predicting the clinical outcome for lung cancer patients.<sup>74–105</sup> As methylated DNA sequences can be found even in the setting of a high background of constitutionally unmethylated normal DNA, they are attractive candidates for early molecular detection tools and for following chemoprevention studies.<sup>108</sup> For instance, one group detected aberrant hypermethylation of *p16* and/or *O6-MGMT* genes in DNA from sputum of patients with squamous cell lung cancer up to 3 years before clinical diagnosis.<sup>109</sup> In addition, abnormal identically methylated DNA can be detected in serum from patients where the tumour was

methylated.<sup>110</sup> Apart from potential diagnostic usefulness, the ability for hypermethylation to be reversed with drugs could lead to therapeutic developments.<sup>111</sup> For instance, retinoic acid plays an important role in lung development and differentiation, acting primarily via nuclear receptors encoded by the retinoic acid receptor-β (*RARB*) gene. *RARB* is often hypermethylated in lung cancers, particularly SCLC,<sup>79</sup> and chemical demethylation may provide an avenue for the re-expression of *RARB*.

#### Limitless replicative potential: telomerase

Telomerase is the enzyme that adds hexameric TTAGGG nucleotide repeats onto the ends (telomers) of chromosomal DNAs to compensate for losses that occur with each round of DNA replication. Normal somatic cells do not have telomerase activity and stop dividing when the telomeric ends of at least some chromosomes have been shortened to a critical length. Immortalised cells, including nearly all lung cancers, probably continue to proliferate indefinitely because they express telomerase.<sup>112–114</sup> While activation of telomerase is not the earliest step in the pathogenesis of lung cancer, it does occur early enough to be a potential molecular marker that can be detected in preneoplastic cells of the bronchial epithelium and in bronchial lavage specimens.<sup>115–116</sup> Because all lung cancers express telomerase, studies of the level of expression in individual tumours will need to be correlated with prognosis and appear to correlate with the presence of lymph node metastases.<sup>117</sup> Besides its use as a diagnostic tool, drugs targeting telomerase have therapeutic potential. Several of these involving anti-sense approaches are nearing entry into clinical trials.<sup>118</sup>

#### Sustained angiogenesis

Lung cancers engender angiogenesis, and the expression of a large number of tumour blood vessels as manifest by tumour microvasculature counts is generally associated with a poor prognosis, although there are some dissenting opinions.<sup>119–122</sup> There are several isoforms of vascular endothelial growth factor (VEGF). The expression ratio of the VEGF189 mRNA isoform had a greater correlation with tumour angiogenesis, postoperative relapse time, and survival than those for the VEGF121, VEGF165, and VEGF206 mRNA isoforms, which suggests that it could be used as a prognostic indicator for patients with NSCLC.<sup>123</sup> This increase in tumour neovascularity arises largely because of production of VEGF by lung cancer cells.<sup>124–126</sup> Part of this dysregulation may arise through loss of p53 function.<sup>126–127</sup> Clinically, plasma VEGF levels can predict the degree of angiogenesis in NSCLC.<sup>128</sup> Some impressive results have recently been presented in abstract form from clinical trials targeting VEGF with a humanised monoclonal anti-VEGF antibody. These initial trials were fraught with toxicity related to unexpected bleeding from large necrotic lung tumour masses, but this should be approachable by patient selection.

#### Tissue invasion and metastases

Many of the changes discussed above lead to the ability of lung cancer cells to invade into tissues and to spread and survive in metastatic deposits.<sup>54–129</sup> One of the interesting new candidates to participate in invasion and metastasis is CRMP-1, a protein involved in mediating the effect of collapsins.<sup>130</sup> Lung cancer specimens showed that reduced expression of CRMP-1 is associated with advanced disease, lymph node metastasis, early postoperative relapse, and shorter survival, indicating that CRMP-1 is involved in cancer invasion and metastasis.<sup>130</sup> Collapsins are part of the semaphorin family, so CRMP-1 may provide another indication of the role of semaphorins and the pathways they mediate in the pathogenesis of lung cancer. Laminins and integrins are being intensively studied as key markers of tissue invasion through

the basement membrane and subsequent development of metastases. The expression of laminin  $\alpha$  chains ( $\alpha 3$  and  $\alpha 5$ ) is often reduced in lung cancer cells; this might contribute to basement membrane fragmentation and subsequent proliferation of stromal elements, as well as having a role in the process of cancer cell invasion.<sup>131</sup> The *LAMB3* gene (encoding the laminin  $\beta 3$  chain, a unique component of laminin-5) was expressed in NSCLC cells and not in SCLC cells.<sup>132</sup> Laminin-5 is a heterotrimeric protein consisting of the  $\alpha 3$ ,  $\beta 3$ , and  $\gamma 2$  chains, and another unique component of laminin-5, the  $\gamma 2$  chain encoded by the *LAMC2* gene. Since  $\alpha 6\beta 4$ -integrin, the specific laminin-5 binding receptor, is known to be expressed only in NSCLCs and not in SCLCs, it appears that laminin-5 is a critical microenvironmental factor for the growth of NSCLC but not of SCLC cells.<sup>132</sup> Survival analysis revealed that overexpression of laminin-5 was associated with shorter patient survival and was an independent prognostic factor in NSCLC.<sup>133</sup>

### GENETIC PREDISPOSITION TO DEVELOPING LUNG CANCER: MOLECULAR EPIDEMIOLOGY

From studies in twins it is clear that there are genetic factors related to smoking initiation and persistence.<sup>134–135</sup> One of the major components of this is therefore nicotine addiction, and a major new field of research is the identification of genetic factors and polymorphisms that predispose to nicotine addiction. Possible associated genes include cytochrome P450 subfamily polypeptide 6 (*CYP2A6*), dopamine D(1), D(2), and D(4) receptors, dopamine transporter, and serotonin transporter genes.<sup>136</sup> Approximately 11% of tobacco smokers ultimately develop lung cancer, which suggests that genetic factors may influence the risk for lung cancer among those who are exposed to carcinogens.<sup>137</sup> Epidemiological studies show an approximately 14-fold increased risk for lung cancer among average tobacco smokers and a 2.5-fold increased risk attributable to a family history of lung cancer after controlling for tobacco smoke.<sup>137</sup> While a rare autosomal dominant gene may explain susceptibility to early onset lung cancer, this would only explain a few lung cancer patients with a family history of lung or other cancers.<sup>137</sup> For example, relatives of retinoblastoma sufferers carrying germ line RB mutation are about 15 times more likely to die from lung cancer than the general population, providing an example of genetic predisposition to lung cancer.<sup>138</sup> However, more common genetic polymorphisms which occur frequently in the population are more likely to have a larger quantitative effect on the risk of lung cancer.<sup>137</sup> The carcinogenesis of tobacco smoke is a process that involves activation of procarcinogens that lead to adduct formation and possible failure of DNA repair which should normally remove these adducts, and polymorphisms which affect each of these steps can be inherited.<sup>137</sup> A lot of research has therefore been directed at identifying the more common genetic polymorphisms which could affect the risk of lung cancer, such as those dealing with molecules associated with carcinogen handling and DNA repair.<sup>137</sup> Studies comparing DNA repair capacity in newly diagnosed lung cancer patients and age matched controls indicate significant differences between the two groups.<sup>137</sup> Polymorphic variation in the activity of enzymes implicated in the metabolism of tobacco carcinogens include the GST family<sup>139</sup> and P450 enzymes.<sup>140</sup> It is therefore likely that individual risk depends on cumulative tobacco exposure and is modified by host genetic differences. Some studies suggest that women have a greater increased risk than men of developing lung cancer following exposure to cigarette smoke carcinogens.<sup>141</sup> In addition, there is recent evidence from Taiwan that women can develop adenocarcinomas of the lung related to oncogenic human papilloma virus

infection.<sup>142</sup> The ultimate relevance of these molecular epidemiology studies will require careful consideration of the relative role of multiple low penetrant genes in determining individual lung cancer risk and translation of this knowledge to the practical fight against lung cancer.<sup>143</sup>

### PRENEOPLASIA AND EARLY DETECTION

In a multistep fashion, preneoplastic cells somatically accumulate genetic alterations which ultimately progress to invasive cancer by clonal expansion. Morphologically distinct preneoplastic bronchial epithelial changes (hyperplasia, metaplasia, dysplasia, and carcinoma in situ) can be observed before invasive cancer develops and are best described for squamous cell carcinomas.<sup>12–66</sup> Mutations (G–A transversions) in genes such as the p53 gene characteristic of cigarette smoke carcinogens are found not only in lung tumours but also in associated non-tumour lung tissue, indicating specific genetic damage by these carcinogens.<sup>144</sup> However, other potential premalignant lesions are now implicated: atypical adenomatous hyperplasia as a precursor for peripheral adenocarcinomas and diffuse idiopathic neuroendocrine cell hyperplasia for carcinoids. Analysis of microdissected preneoplastic lesions suggests one model of sequential LOH at chromosome regions 3p, then 9p, 8p, 17p (with p53 mutation), 5q, and RAS mutations, although it is likely that there is some molecular heterogeneity as exists in overt cancers.<sup>12–66 145–148</sup> The genetic changes found in invasive cancers and preneoplasia can also be identified in morphologically normal bronchial epithelium from current or former smokers.<sup>11 12 148–151</sup> Some reversal of the morphology can occur after smoking cessation, although the increased risk of lung cancer does not completely return to baseline, raising the possibility of irreversible damage. These observations are consistent with “field cancerisation”, whereby the whole tissue region is repeatedly exposed to tobacco smoke and is at risk of developing multiple, separate, clonally unrelated foci of neoplasia, a notion supported by the widespread presence of aneuploidy in the respiratory tree of smokers.<sup>152</sup> This was confirmed in a study of 195 histologically normal or slightly abnormal epithelium samples and 23 dysplastic epithelium samples from 19 lobectomy specimens where two thirds of the 19 lobectomies had at least one focus of molecularly altered bronchial epithelium.<sup>13</sup> The findings indicated that multiple small clonal or subclonal patches containing molecular abnormalities are present in normal or slightly abnormal bronchial epithelium of patients with lung cancer.

### FUTURE RESEARCH

How do we exploit this knowledge and the technological advances in, for example, the ability to obtain and test cells and cellular products such as DNA and RNA from sputum, bronchial biopsy specimens, brushing specimens, lavage fluids, and blood? A major challenge is to find a biomarker or panel of markers with sufficient sensitivity and specificity for detecting the variety of lung cancer types, given their molecular heterogeneity. In this regard, acquired hypermethylation changes may be helpful as a panel of biomarkers that will be able to detect abnormalities in most lung cancers. However, a potential biomarker needs not only to be easily detectable in body fluids (that is, sensitive), but it must also be specific for neoplastic transformation and not just reflect smoking related lung damage. Consider, for example, the phenomenon of microsatellite alterations (a PCR method of detecting acquired qualitative and quantitative changes in microsatellite repeat sequences) which have now been found in patients with chronic obstructive pulmonary disease as well as in patients with lung cancer.<sup>153 154</sup>

**Table 1** Clinical use (translation to the clinic) of molecular information in lung cancer

Issue	Potential approaches
Molecular epidemiology	Genetic stratification of lung cancer risk; integration of this assessment into smoking cessation treatments, chemoprevention, and screening trials
Early detection	Detection of lung cancer specific genetic and epigenetic changes in sputum, blood, bronchial biopsies, brushings, and lavage specimens
Chemoprevention	Determination of the genetic and epigenetic abnormalities in specimens from bronchial epithelium to use as intermediate biomarkers for response to chemoprevention and smoking cessation
Diagnosis	Use of biomarkers as an adjunct to standard morphological and histological diagnosis and to assist in subtype differentiation
Treatment	Direct treatment at molecular targets abnormal in lung cancer but not in normal tissues. These include inhibitors of oncogenes, growth factors and their receptors such as tyrosine kinase inhibitors, farnesyltransferase inhibitors, cyclin dependent kinase inhibitors, cyclo-oxygenase inhibitors, antisense molecules, replacement gene therapy to correct tumour suppressor gene defects, apoptosis modulators, and inhibitors of angiogenesis and telomerase.
Prognosis	Use of molecular biomarkers to predict outcome including survival, metastatic potential, and probability of response to chemotherapy, radiotherapy or biological treatments

## CONCLUSIONS

It has been hypothesised that clinically evident lung cancers have accumulated many different genetic or epigenetic abnormalities in oncogenes and/or TSGs. This notion has important clinical ramifications; it should, for instance, be possible to discover carcinogen exposed bronchial epithelial cells with only a subset of these changes and to intervene with very early treatment and/or chemoprevention. The finding of some commonality in the molecular lesions in this heterogeneous group of malignancies recognised histologically as lung cancer is also important, with implications for developing specific diagnostic and therapeutic biotargets, summarised in table 1. The intrinsic molecular heterogeneity will pose problems for designing global approaches that would be suitable for all lung cancers but, conversely, may provide an insight into how different lung cancer subtypes hijack biological processes in their attempt to evolve and grow. The ability to detect molecular changes in at risk bronchial epithelium may translate into novel intermediate markers for chemoprevention. One of the biggest challenges will be integrating the molecular based strategies such as biomarkers or treatment options with conventional lung cancer management tools; we will need to learn how to exploit early detection biomarkers with modern imaging such as low dose helical CT scanning and targeted treatments with conventional chemotherapy and radiotherapy.<sup>155</sup> An early example of this is the use of  $\beta$ -tubulin mutations to predict the response to paclitaxel.<sup>156</sup> Overall, these translational studies need to link the laboratory with the pulmonary physicians who care for patients with thoracic disease and with those involved in screening trials for lung cancer using, for example, spiral CT scans.<sup>157</sup> Finally, these studies need to be integrated with investigation of a genetic predisposition to lung cancer arising from polymorphisms in the human population.<sup>137-158</sup>

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## LUNG ALERT .....

### Airway stents provide symptomatic relief

▲ Wood DE, Liu Y, Vallières E, *et al*. Airway stenting for malignant and benign tracheobronchial stenosis. *Ann Thorac Surg* 2003;**76**:167–74

**T**his paper reports the indications, techniques, and results of tracheobronchial stenting in the authors' unit. One hundred and forty three patients underwent 309 stent procedures between May 1992 and December 2001. Malignancy was the most common indication (67% of cases). The main symptoms were dyspnoea, respiratory distress, and stridor. 82% of patients required emergency procedures as most (77%) had more than 75% narrowing of the airway. All patients underwent rigid bronchoscopies except two who had flexible bronchoscopies for insertion of expandable metal stents. 87% of stents placed were silicone rubber. 27% of patients had primarily a tracheal obstruction but in 49% multiple sites were involved. 15% required multiple stents and 68% had a bronchoscopic intervention (core out, dilatation, brachytherapy, laser or photodynamic therapy) in preparation or as an adjunct to stenting. Significant improvement was reported in 95% of patients; 41% required multiple bronchoscopies to maintain this improvement. Complications from stenting occurred in 41% of cases—namely, stent migration, partial occlusion of stent by secretions, and partial obstruction by granulations. Perforation occurred in four patients but only one required thoracotomy. 28 % of patients with malignancy required further intervention to maintain airway patency (mean airway palliation 4 months). 45% of patients with malignant disease and 17% with benign disease had no follow up data available following the original intervention.

This paper shows that stenting can produce immediate symptomatic improvement in unresectable patients but multiple stents and procedures are frequently required.

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