Application of Molecular Genetics to the Early Diagnosis and Screening of Lung Cancer

Michael J. Birrer and Powel H. Brown

Biomarker and Prevention Research Branch, Division of Cancer Prevention and Control, National Cancer Institute, and Uniformed Services University of The Health Sciences, Naval Hospital Bethesda, Bethesda, Maryland 20814

Abstract

Recent studies of the molecular biology of lung cancer have identified multiple abnormalities. Despite this vast cataloging of genetic lesions, the chronology of these events and those which occur early remains essentially unknown. This review summarizes the genetic abnormalities in lung cancer cells, including mutation, amplification, and overexpression of dominant protooncogenes as well as deletion and mutation of recessive oncogenes. In addition, possible candidate genes exist which may participate in the early activation events of lung cancer, and evidence for their role in the early development of cancer is discussed. These lesions may be helpful in developing strategies to screen for lung cancer.

The characterization of the molecular events which lead to the transformation of bronchial epithelial cells into invasive lung cancer cells may provide effective tools for the early detection and diagnosis of lung cancer. As initially suggested by the results of model systems (1), and more recently supported by studies of the molecular pathogenesis of colon cancer (2), a multistep model of epithelial carcinogenesis has evolved. It is now apparent that multiple genetic changes including mutations, deletions, gene amplification, or translocations occur during the transformation of a normal cell to a malignant one. Most human cancers, including lung, breast, colon, pancreatic, stomach, skin, bladder, ovarian cancer, and lymphomas and leukemias, have been found to have genetic changes in multiple target genes (3-11). In this paper, we will review the molecular genetics of lung cancer, including early activation events, and will discuss potential ways of exploiting this information to impact on this disease. By identifying these molecular events and their chronological sequence, more effective screening techniques can be developed which may allow the early detection and diagnosis of bronchial dysplasia or carcinoma-in-situ, which in turn may lead to effective prevention or treatment of invasive carcinoma, ultimately improving the outcome of patients with this presently highly lethal cancer.

Dominant Oncogenes

Early studies of carcinogenesis revealed that certain genes could induce cellular transformation (12-14). These genes when activated by mutation lead to deregulated cellular proliferation. Such genes, now called dominant oncogenes or protooncogenes, have been found to encode proteins which function as growth factors, growth factor receptors, signal transducing proteins, and nuclear proteins involved in transcriptional regulation (reviewed in Ref. 15). Amplification of these genes, mutations within the genes, and mutations or DNA translocations which affect these genes' promoters have been documented in many different human cancer cells and have been shown to lead to gene activation or overexpression (15). Such mutations have been documented in primary lung cancer cells and cell lines, suggesting that the activation of dominantly acting oncogenes is an important step in the cascade of events which ultimately leads to the development of invasive cancer (see Table 1).

Mutations within the genes of the ras family of oncogenes are one of the genetic abnormalities identified in lung cancer cells. This family comprises a group of membrane-associated GTP-binding proteins which are felt to be involved in signal transduction. Members of this family include the closely related N-ras, K-ras, and H-ras oncogenes and protooncogenes, as well as genes coding for more distantly related GTP-binding proteins such as the ras and rho and the rab and rap gene families (rab1 through rab4, and rap1 and rap2) (reviewed in Refs. 16 and 17). Many different human cancers have been found to have "activating" point mutations within the ras genes (5). Such point mutations occur in codons 12, 13, and 61 of the K-ras, N-ras, and H-ras genes. These "activating" mutations induce structural changes within the ras protein leading to an "activated" GTP-bound conformation.

Direct nucleic acid sequencing of the members of the ras gene family has revealed that such activating mutations occur in 15 to 30% of human NSCLC, most of which are adenocarcinomas (18-22). In an early study, Rodenhuis et al. (18) found K-ras mutations in 5 of 35 primary lung cancer cells. All of the tumors with activated ras genes were adenocarcinomas. In a subsequent study (19), this same group reported activated ras genes in 9 of 35 primary adenocarcinoma lung tumors. Mitsu- domi et al. (20) reported finding ras mutations in 22 of 65 NSCLC cell lines (predominantly K-ras mutations in adenocarcinomas, but they also rarely found mutations in H-ras and N-ras in cells of other histologies). Of note, no ras mutations have been found in human small cell lung carcinoma. Recent studies from two different groups have suggested that such ras mutations may have prognostic significance. Slebos et al. (21) studied the ras mutations in lung cancer cells from patients with early stage adenocarcinoma who underwent potentially curative reection of their tumors. They found a highly significant decrease in both disease-free survival and overall survival in the patients whose tumors had mutations within the K-ras gene. This finding has been recently confirmed by Mitsuodomi et al. (22) who also studied ras genes from 66 NSCLC cell lines from patients with both early and late stage NSCLC. This group found that patients whose tumors had K-ras, N-ras, or H-ras mutations had decreased survival compared with patients whose tumors had no ras mutations. This decrease in survival was seen in patients with either limited or advanced disease. In light of this evidence that mutations within ras genes may have prognostic significance in patients with NSCLC, it may be reasonable to consider additional adjuvant therapy in patients who have early stage resectable tumors whose cells are found to have ras mutations. In addition, careful screening of the ras gene or its protein product in bronchial epithelial cells may identify patients with early aggressive tumors who might require more aggressive therapy than standard surgical resection.

Another family of protooncogenes, the erb B family, has been found to be abnormally expressed in lung cancer cells. This

2 To whom requests for reprints should be addressed.

The abbreviations used are: NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; SCLC, small cell lung cancer; RFLP, restriction enzyme fragment length polymorphism(s); SV40, simian virus 40; cDNA, complementary DNA; PCR, polymerase chain reaction.

2658s
family of protooncogenes contains erb B1, the gene coding for the EGFR, and erb B2 (also called Her-2/neu), a closely related gene. The erb B1 oncogene was initially discovered as one of two oncogenes carried by the avian erythroblastosis virus (23). The corresponding protooncogene was found to encode a membrane-associated tyrosine kinase protein which was eventually identified as the receptor for EGF (24-26). The other member of this gene family, the erb B2 protooncogene also encodes a membrane-bound tyrosine kinase, felt to be a receptor for an as yet unidentified growth factor.

The erb B1 or EGFR gene has been found to be amplified in NSCLC (up to 20% of squamous cell tumors) (31-33), while the erb-B1 protein, the EGF receptor, has been shown to be overexpressed in many NSCLC cells (approximately 90% of squamous cell tumors, 20 to 75% of adenocarcinomas, and rarely in large cell or undifferentiated tumors) (32, 34, 35). Of note, erb B1 gene amplification or protein overexpression has not been seen in small cell lung cancer cells. The overexpression of the EGF receptor may reflect the development of an autocrine growth loop, as EGF is required by most epithelial cells, including NSCLC cells, for their growth (36). These findings have led to plans for the development of clinical trials using antibodies directed against the EGF receptor in the treatment of NSCLC (37).

The erb B2, or Her-2/neu, protooncogene has also been found to be amplified in many human tumors including breast, stomach, kidney, and lung cancers (31, 33, 38-41). erb B2 gene amplification is seen in lung cancer cells and cell lines, predominantly in adenocarcinoma; however, this occurs much less frequently than does erb B1 gene amplification. Overexpression of the erb B2 protein product has also been observed in all histologies of NSCLC cells. Recent data showing decreased survival (independent of stage) in patients with adenocarcinoma whose cancer cells overexpressed the erb B2 protein suggest that the level of erb B2 protein expression may be a prognostic indicator in these patients (42). In addition, low levels of the erb B2 protein are seen on the “normal” bronchial epithelium of patients with lung cancer (42-44).

Members of a third family of dominantly acting oncogenes, the myc family, which includes the c-myc, N-myc, and L-myc genes, are also abnormally expressed in lung cancer cells. c-myc was first identified as the cellular homologue of the transforming gene in the avian myelocytomatosis virus (45). The other myc family members were discovered by virtue of their sequence homology to c-myc, with L-myc being identified as an amplified gene in a human SCLC cell line (46). These genes encode nuclear phosphoproteins, which have potent effects on cell growth and which may function as transcriptional regulators. Recent data (47, 48) suggest that these proteins can complex with another nuclear protein called Max to form an active dimer which can bind DNA and may activate transcription.

Unlike ras genes, which are activated by point mutations in lung cancer cells, the myc genes are “activated” by overexpression of the cellular myc genes, either by gene amplification or by deregulated transcription (3), each ultimately leading to increased levels of myc protein (49, 50). To date, no coding sequence mutations have been found in myc genes in human cancer. Amplification of the normal myc genes is seen frequently in SCLC (51-56), and rarely in NSCLC (51, 57), and has been found to involve c-myc, N-myc, and L-myc. Of note, only one myc family member is amplified in any given tumor specimen or cell line. No examples of two family members being overexpressed in the same tumor have been found.

How myc overexpression is related to the transformation of human lung cancer cells is presently unknown. However, in model systems overexpression of c-myc, N-myc, or L-myc can complement an activated ras gene to transform primary rat embryo cells (58-61). In addition, introduction of the c-myc gene into classic SCLC cells, with subsequent overexpression of the c-myc protein, leads to a phenotypic change, producing cells with a typical “variant” SCLC morphology which proliferate more rapidly and clone more efficiently in soft agar (62). Clinically, myc gene amplification is correlated with more aggressive tumors and decreased patient survival (52, 53, 56, 63), consistent with the hypothesis that lung cancer cells which have deregulated myc expression are more rapidly growing and more aggressive. Thus, the deregulation and overexpression of myc genes may be an important step in the development or progression of SCLC.

Other dominant oncogenes found to be expressed at high levels in lung tumors include members of the raf gene family (c-raf-1) (64) and the jun gene family (Ref. 65; Footnote 4); however, these protooncogenes are also expressed in normal lung tissue, and therefore their role in the pathogenesis of lung cancer is unclear. The src-related tyrosine kinase genes, src and lck, have also been shown to be expressed in lung cancer cell lines (54, 66). In addition, one group has suggested that src kinase activity correlates with neuroendocrine differentiation of small cell lung cancer (67).

### Table 1 Oncogenes involved in lung cancer

<table>
<thead>
<tr>
<th>Oncogenes</th>
<th>Mechanism</th>
<th>Histology</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ras family (H-ras, N-ras, K-ras)</td>
<td>Point mutation</td>
<td>NSCLC</td>
<td>18-22</td>
</tr>
<tr>
<td>myc family (c-myc, L-myc, N-myc)</td>
<td>Gene amplification, overexpression</td>
<td>SCLC</td>
<td>51-57</td>
</tr>
<tr>
<td>erb B family (erb B1, erb B2/Her-2/neu)</td>
<td>Gene amplification, overexpression</td>
<td>NSCLC</td>
<td>31, 33, 38, 41</td>
</tr>
<tr>
<td>Retinoblastoma gene (Rb)</td>
<td>Deletion, point mutation</td>
<td>SCLC, NSCLC</td>
<td>75, 76</td>
</tr>
<tr>
<td>p53 gene</td>
<td>Point mutation</td>
<td>SCLC, NSCLC</td>
<td>4, 8, 92-95</td>
</tr>
<tr>
<td>3p gene(s) (unidentified)</td>
<td>Deleted</td>
<td>SCLC</td>
<td>96</td>
</tr>
<tr>
<td>raf genes (c-raf-1)</td>
<td>Expressed</td>
<td>SCLC, NSCLC</td>
<td>64</td>
</tr>
<tr>
<td>jun family (c-jun, jun B)</td>
<td>Expressed</td>
<td>SCLC, NSCLC</td>
<td>65</td>
</tr>
<tr>
<td>src family (src, lck)</td>
<td>Expressed</td>
<td>SCLC, NSCLC</td>
<td>54, 66</td>
</tr>
<tr>
<td>Retinoic acid receptor genes (RARA, RARb, RARy)</td>
<td>Deleted?</td>
<td>SCLC</td>
<td>97</td>
</tr>
<tr>
<td>Phosphatases (phosphotyrosine phosphatase)</td>
<td>Deleted?</td>
<td>SCLC</td>
<td>98</td>
</tr>
</tbody>
</table>

4 Unpublished observations.
Recessive Oncogenes

Analysis of the molecular genetics of multiple human cancers has demonstrated that the loss or inactivation of certain genes may also be important steps in the pathway leading to invasive cancer. Such genes have been called "tumor suppressor genes," as they may function normally to suppress cellular proliferation, or "recessive oncogenes," as mutations or deletions must occur in both alleles of these genes before transformation occurs (reviewed in Ref. 68). One clue to the existence of such tumor suppressor genes has been the detection of nonrandom chromosomal loss in many tumors by cytogenetic analysis. Such chromosomal abnormalities have been associated with RFLP which correlate with the deletion of one allele of a putative tumor suppressor gene. In cases where the specific tumor suppressor gene is identified, smaller deletions or point mutations are also found in the remaining allele (69, 70), leading to functional inactivation of the tumor suppressor gene and the development of invasive cancer.

The first such tumor suppressor gene described was the retinoblastoma gene (Rb), identified after classical genetic studies by Knudson (71) predicted that inactivation of both alleles of a gene led to the development of childhood retinoblastoma. This gene has been cloned, mapped to the long arm of human chromosome 13 (72, 73), and subsequently been found to be homozygously lost or mutated in most retinoblastoma cells (72–74). In addition, deletion or mutation of this same gene has been described in osteosarcoma (72, 73), small cell lung cancer (75, 76), bladder cancer (76), and rarely in breast carcinoma cells (76–78). The Rb gene product is a Mr 110,000 phosphoprotein which undergoes cell cycle-dependent phosphorylation and is thought to be involved in cell cycle regulation (68). In addition, the Rb protein binds to a variety of DNA binding proteins including viral oncoproteins such as SV40 large T-antigen, adenovirus E1A, and papilloma virus E7 (reviewed in Ref. 79), as well as multiple as yet uncharacterized endogenous cellular proteins (80). This association with DNA binding proteins is thought to be critical for the normal function of the Rb protein, as mutations in the Rb gene which are detected in human tumors frequently produce protein products which fail to associate with the Rb-binding proteins mentioned above (81, 82).

Cytogenetic analysis of human lung cancer cells has revealed nonrandom chromosomal abnormalities of chromosomes 1, 3, 13, and 17 (83). Subsequent study of the Rb gene (located on chromosome 13) in SCLC primary tumors and cell lines revealed that the Rb gene is deleted or mutated in 13% and 18% of these cells, respectively (75). A more detailed analysis of the Rb DNA, RNA, and protein showed that Rb abnormalities occur at some level in greater than 95% of SCLC and 20% of NSCLC cells (76).

Another tumor suppressor gene, p53, has been mapped to the short arm of chromosome 17. The p53 gene was originally identified as a nuclear protein which bound to the large T-antigen of the SV40 DNA tumor virus (84, 85). While this gene was initially felt to act as a dominant oncogene, further investigation indicated that a mutant form of p53 was being studied (86). When the wild-type p53 gene was tested for its ability to transform cells, it was discovered that wild-type p53 could suppress transformation, while a mutant form of p53 could induce transformation (86). The p53 protein is known to bind to viral DNA-binding oncoproteins (such as SV40 large T, adenovirus E1B, and papilloma virus E6) (87), and recent studies now suggest that the p53 protein may function by regulating DNA transcription (88–90). Loss or mutation of the p53 protein may lead to abnormal gene expression and ultimately deregulated cell growth. The recent discoveries of p53 mutations in colon (7, 8), breast (8, 91), brain (8), and lung cancer cells (4, 8, 83, 92), as well as in the Li-Fraumeni syndrome (93), support this hypothesis and suggest that the wild-type p53 protein may function in many different cells to suppress transformation.

The observation that deletion of the short arm of chromosome 17 is frequently seen in lung cancer cells led to the study of p53 gene expression in these cells. Direct DNA sequencing of the p53 gene from freshly resected early stage NSCLC tumors revealed p53 point mutations in 42% of the specimens (94). In a similar study of p53 in SCLC cell lines, D'Amico et al. (95) found that 100% of the SCLC cells had p53 mutations. Many of the different mutant p53 proteins have a prolonged half-life and can be detected histochemically, while wild-type p53 is undetectable using this technique. This has allowed histochemical staining to be used as a screening test for p53 mutations. In such a histopathological study, Iigo et al. (92) have shown that 55% of resected lung cancers have detectable p53. Direct sequencing of the p53 cDNA from three of the tumors which stained with antibodies specific for p53 confirmed the presence of a p53 point mutation, while sequencing of the cDNA from a tumor which failed to stain revealed wild-type p53. Thus, p53 point mutations are frequent events in both SCLC and NSCLC cells, a finding supporting the hypothesis that mutations in this gene may be an important step in the transformation of bronchial epithelial cells into carcinoma.

As mentioned above, another common cytogenetic abnormality in lung cancer cells is the loss or deletion of the short arm of chromosome 3 (3p). RFLP analysis has revealed that chromosome 3p is the most common site to show loss of heterozygosity (indicating the loss of one of the alleles of the restriction fragment being tested) in lung cancer cells, with 90 to 100% of SCLC cells and 25 to 50% of NSCLC cells showing deletion of this region of chromosome 3 (96). Such a deletion, coupled with a mutation within a putative tumor suppressor gene located on chromosome 3p, could be another important step in the development of lung cancer. These findings have led multiple laboratories to search for genes which map to chromosome 3p and are deleted or mutated in lung cancer cells. To date, such tumor suppressor genes remain elusive (but see below).

Other candidate tumor suppressor genes include the retinoic acid receptors (RARs), DNA binding proteins which regulate gene transcription (97), and the recently described tyrosine phosphatases (98), which may function to balance the action of tyrosine kinases, some of which are known dominant oncogenes, to control cell growth. These genes are particularly interesting as the genes for RARs and phosphorylases both map to chromosome 3p and may ultimately be found to be the tumor suppressor genes on chromosome 3p which are frequently lost in SCLC cells.

Early Activation Events

While the cataloging of molecular genetic lesions in lung cancer has proceeded briskly, determination of the chronology of these events is only in its infancy. It is likely that many of these well-characterized genetic lesions occur late as "progression" events contributing to the invasive and metastatic prop-
properties of tumors and less to their establishment. Unfortunately, at present, the early activation events in lung cancer remain a mystery. Clues for these events can be found in in vivo animal model systems and in inherited human cancers, which suggest that mutations in certain key genes can function under appropriate conditions as "initiation" events for the development of cancer. Included among these are the dominant oncogenes, ras and Her-2/neu, and the recessive oncogenes, Rb and p53 (Table 2). Therefore, by extrapolation, these genes are reasonable candidates for a role in early lung cancer and warrant further analysis. In addition, these genes and their mutations are particularly amenable to analysis, since they can be characterized from minute amounts of tissue found in preneoplastic lesions.

In mouse carcinogenesis model systems (primarily skin and mammary), exposure of the animals to a combination of carcinogens and tumor promoters results in high frequency tumor formation (99–101). These tumors uniformly contain activated ras genes and, more importantly, one can identify these ras mutations prior to the gross appearance of a tumor (101). Furthermore, this carcinogenic induced "initiation" event can be replaced by infection of these mice with a retrovirus carrying an activated ras gene (102). In these infected animals, all cells possess this viral activated ras gene as the "initiating" event and proceed to give rise to tumors at very high rates. In human tumors, there is a paucity of data concerning the possibility of activated ras genes occurring early in disease states. Two examples do exist; myeloproliferative syndromes have been found to contain activated N-ras genes and, mutations in K-ras genes have been identified in adenomatous polyps of the colon (103, 104).

As described above, Her-2/neu is a tyrosine kinase which is expressed at low levels in the bronchial epithelium but can be found to be overexpressed in a subset of non-small cell lung cancer (41–43). It is presently unknown at which point this gene plays a role during the development of lung cancer. Evidence that it can function as an "initiating" event comes from an in vivo model system using the Her-2/neu gene in transgenic mice (105). This gene, under the control of a steroid-inducible promoter, produces transformation of all mammary cells in the breasts of these animals, suggesting that this gene could function well as an "initiating" event.

The timing of the genetic lesions identified in recessive oncogenes during the development of lung cancer also remains unknown. However, in contrast to dominant oncogenes, there exists substantial evidence that these genes can function early in the development of cancer. Both the p53 and Rb genes are involved in well-characterized genetic syndromes where individuals inherit mutations and are at high risk for the development of tumors (69, 93). The Li-Fraumeni syndrome, which is characterized by the development of multiple different tumors at early ages, has now been shown to result from the inheritance of a mutated p53 gene. In addition, the familial form of retinoblastoma results from the inheritance of a mutated retinoblastoma gene. In this form of the disease (in contrast to the sporadic form), tumors occur early and are bilateral and multifocal. Thus, these two recessive oncogenes can clearly function as early activation events when inherited, which suggests that they may serve this purpose more generally in other tumors. In addition, inactivating mutations in the p53 gene have been identified in a truly preneoplastic lesion. Casson et al. (106) have reported finding mutations in the p53 gene in Barrett's esophagus, a precursor to adenocarcinoma of the esophagus. Whether such a relationship exists for lung cancer remains to be determined.

Well-known genes, such as the members of the myc, jun, and fos gene families, and other as yet uncharacterized genes, such as the putative recessive oncogene(s) on 3p, may also play important roles in the early development of lung cancer. While we have focused on genes for which there are some compelling data supporting their role in early activation events and which are amenable for analysis as early markers of transformation, further work will need to be done on these less characterized genes. In addition, it should be noted that there may be no single unique sequence of molecular events which gives rise to lung cancer but multiple different combinations, any one of which is capable of producing full malignant transformation. As such, no single genetic lesion, but perhaps a limited number of lesions, may function as activation events for lung cancer.

Use of Early Activation Events as Molecular Markers for Early Detection

Although early activation events for lung cancer have not been delineated, the identification of potential candidate genes (as described above), coupled with new technologies for characterizing them from relatively small amounts of tissue, will provide an opportunity for determining their precise role in neoplastic and/or preneoplastic lesions of the lung and for potentially using them as targets for screening assays.

The PCR allows for the amplification of specific DNA from small amounts of tissue and potentially even single cells (107, 108). PCR amplification of ras genes has been accomplished and reported by multiple laboratories. This technique when applied to preneoplastic lung epithelium, such as dysplastic or metaplastic lesions, may answer whether activated ras genes play a role early in lung cancer. Amplified sequences can be analyzed to determine the presence of mutations by several methods: (a) differential hybridization of 32P-labeled mutated oligonucleotides (109); (b) identification of new restriction enzyme sites created by the activating mutations (110); (c) single-strand conformational polymorphisms (111); and (d) nucleic acid sequencing. These methods combined with PCR technology could allow detection of an activated ras gene from sputum specimens. This may provide a potential screening test for high-risk patients, such as patients with resected Stage I lung cancer, heavy smokers, or patients with head and neck tumors. Limitations of this approach include nonspecific amplifications,
false positive mutations, and the fact that only 30% of adenocarcinomas (15% of all lung tumors) of the lung have activated ras genes.

As noted above, the Her-2/neu gene is overexpressed in a subset of non-small cell lung cancers. This abnormality is not associated with a specific protein mutation. Previous studies have suggested that the best method to detect the presence and overexpression of this gene has been through the use of high titered antiserum made against the protein product. This antiserum has detected high levels of the Her-2/neu protein product in breast and ovarian cancers and, in both cases, high expression was associated with a poor prognosis (39, 40). These techniques and reagents could be applied to early preneoplastic lesions in the lung and ultimately on a single cell basis (using sputum samples) as a screening approach. The major limitations of this approach are the small numbers of tumors in which this oncogene is aberrantly expressed (30%).

The recessive oncogenes p53 and Rb also provide potentially convenient targets for the screening of individuals at high risk for lung cancer. The Rb gene has been found to be deleted or mutated in the vast majority of small cell lung cancer tumors and a small portion of non-small cell lung cancer tumors. Detection of these mutations by PCR amplification and nucleic acid sequencing or through the use of Rb-specific antiserum is possible and applicable to large numbers of samples containing small amounts of tissue. Problems in using Rb as a target gene for screening include its limited role in lung cancer. Its role is limited primarily to small cell lung cancer, which has no known premalignant precursor, and is infrequently diagnosed by sputum cytology. On the other hand, the p53 gene lends itself to easier analysis, and mutations in the p53 gene have been detected in the vast majority of lung cancers. Therefore, p53 may ultimately be a better target for molecular screening for early disease. Potential mutations in this gene can be identified by PCR amplification of the coding regions of the gene and analyzed by direct nucleic acid sequencing or single-strand conformational polymorphism analysis. This approach has already identified p53 mutations in relatively small samples of tissue, such as urine cytologies and “touch preparations” from breast cancer specimens (112). In addition, these techniques can be supplemented or complemented by other approaches, such as immunohistochemical staining, using high titered antiserum directed at wild-type p53 or specific for the mutated form. This technique would allow for the evaluation of small amounts of tissue, such as that found in bronchial biopsies or sputum samples.

Examination of preneoplastic lesions or sputum samples for genetic lesions from patients at high risk for lung cancer is critical to our understanding of the early events of this disease. If any or all of the above genetic mutations are identified as sensitive and specific markers, we may be at a point where we can begin to devise molecular screening techniques for lung cancer. On the other hand, it is conceivable that these lesions will be considerably more common (less specific) than initially thought, consistent with the hypothesis of “field cancerization,” which argues that high-risk patients (such as smokers) have multiple areas of epithelium which have undergone “initiation” events. Only through further study of the nature and sequence of genetic changes leading to the transformation of bronchial epithelium will these questions be answered. If, indeed, these events are found frequently, it may be necessary to use combinations of molecular lesions or overall accumulation of ‘hits,’ compared with the “normal” cells of smokers, to determine the relative risk of lung cancer.

Summary

Although great progress has been made in understanding the molecular genetics of lung cancer, our knowledge of the early activation events remains preliminary. It is now paramount in our understanding of this disease to identify which, if any, of the genetic lesions associated with lung cancer are critical early steps. By applying the techniques of modern molecular biology, it is conceivable that one could devise approaches which will identify early disease in high-risk patients. This may allow us to effectively impact on this deadly disease at an early and more treatable time point.

References


2662s
MOLECULAR GENETICS AND EARLY LUNG CANCER SCREENING


Downloaded from cancerceres.saccournals.org on June 1, 2017. © 1992 American Association for Cancer Research.
MOLECULAR GENETICS AND EARLY LUNG CANCER SCREENING


Application of Molecular Genetics to the Early Diagnosis and Screening of Lung Cancer

Michael J. Birrer and Powel H. Brown