Molecular markers in lung cancer: prognostic role and relationship to smoking*

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ABSTRACT

Epidemiological studies have demonstrated a causal relationship between smoking and lung cancer. Although most lung cancer cases are linked to smoking, only a minority of heavy smokers develop lung cancer, leading to the notion that genetic factors affect individual susceptibility. The principal molecular changes in lung cancer are seen in tumor suppressor genes, proto-oncogenes, growth factors, telomerase activity, and methylation status of promoters. Well-known agents include angiogenesis-stimulating factors (such as vascular endothelial growth factor), as well as factors related to tumor cell proliferation and apoptosis (epidermal growth factor receptor, p53, K-ras, retinoblastoma and BCL-2). Several of these genetic factors have already been investigated, but no single parameter has yet presented sufficient selectivity regarding prognostic value or therapeutic efficacy. Treatment strategies to cure lung cancer should focus on these early genetic lesions in order to promote their repair or to eliminate these lung cancer cells.

Keywords: Smoking; Lung neoplasms; Genetic markers; Prognosis

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INTRODUCTION

Statistically, lung cancer is the leading cause of death from cancer, regardless of gender, in the United States, where it is responsible for approximately 170,000 deaths per year, corresponding to one-sixth of all cancer deaths. Lung cancer affects a greater number of people worldwide (approximately 1.2 million new cases per year) than does any other cancer. Because lung cancer patients are typically diagnosed in the severe stage of the disease, the five-year survival rate is less than 15%. Approximately 80% of all lung cancer cases are non-small cell lung carcinoma (NSCLC): squamous cell carcinoma, adenocarcinoma or large cell carcinoma. The remaining cases are small cell lung carcinoma (SCLC). These distinctions are important in treatment and prognosis.\(^{1-2}\)

In the United States, the incidence of lung cancer stabilized during the 1995-2001 period. However, there was an annual 0.3% incidence increase among women from 1987 to 2001. Among men, the three principal types of cancer are prostate, lung and colorectal, together accounting for 56% of all diagnosed cancers (prostate cancer alone accounting for 33%). Among women, breast, lung and colorectal cancer predominate, together accounting for 55% of all diagnosed cancers (breast cancer alone accounting for 32%).\(^{1-2}\)

The incidence of lung cancer in Brazil has increased in recent decades, and lung cancer mortality remains high, similar to what is seen in the rest of the world.\(^{3-7}\) In Brazil, lung cancer is the leading cause of death from cancer among men and the second leading cause of cancer death among women. The estimated number of new lung cancer cases in Brazil for 2005 is 17,110 among men and 8,680 among women. These values correspond to an estimated risk of 17,110 among men and 8,680 among women. These values correspond to an estimated risk of 19 new cases/100,000 men and 9 new cases/100,000 women. In Brazil, lung cancer is the second leading cause of cancer death among men (after nonmelanoma skin cancer) in the southern (36/100,000), southeastern (23/100,000) and midwestern (15/100,000) regions. In the northern and northeastern regions, lung cancer is the third leading cause of cancer death among men (8/100,000 in both regions). Among Brazilian women, lung cancer is the fourth leading cause of cancer death in the southern (16/100,000), southeastern (11/100,000), midwestern (8/100,000) and northern regions (5/100,000). In the northeastern region, it is the fifth leading cause of cancer death among women (5/100,000).\(^{3-7}\)

SMOKING AS A RISK FACTOR

There are various risk factors for lung cancer, including asbestos, radon, occupational exposure, environmental exposure and genetic factors. However, the most significant factor is smoking, which accounts for 80% of the attributed risk among men and for 45% of the cases among women.\(^{3-7}\) The incidence rates of lung cancer are generally higher among men than among women. It has been observed that the rates among women have been increasing, whereas the rates among men have remained stable, tending to decrease.\(^{3-7}\) Lung cancer remains a highly lethal disease. Mean cumulative five-year survival rates range from 13% to 21% in developed countries and from 7% to 10% in developing countries, with an estimated global mean of 11%.\(^{3-7}\)

The causal relationship between smoking and lung cancer has been accepted since the 1950s, when case-control studies revealed a relative risk of 10.\(^{8-9}\) In cohort studies, it has been demonstrated that lung cancer mortality increases in proportion to the level of smoking, this factor being more significant than the tar and nicotine content of the tobacco.\(^{8-10}\) Epidemiological data on smoking and lung cancer meet causality criteria: consistency of results; correlation strength; specificity; temporal sequence between exposure and outcome; and biological plausibility.\(^{11}\)

Lung cancer incidence rates in a given country reflect levels of tobacco consumption. A house-to-house survey carried out in Brazil revealed that the percentage of regular cigarette smokers ranged from 13% to 25%. The highest percentages were observed in the cities of Porto Alegre, Curitiba and Florianópolis, located, respectively, in the states of Rio Grande do Sul, Paraná and Santa Catarina. A high prevalence of smoking among adolescents was observed (between 9% and 27%), as well as an early mean age at acquisition of the smoking habit, principally among women. These results underscore the importance of antismoking programs, especially among women and youths.\(^{3-7}\)

Approximately 87% of lung cancer cases are tobacco exposure-related. The relative risk of developing lung cancer is 24 times higher among smokers than among nonsmokers. The relative risk
of lung cancer for former smokers, albeit lower than for smokers, is higher than for nonsmokers, reinforcing the need for prevention measures since over 50% of lung cancer cases occur among former smokers. The use of chemoprevention in former smokers relies on the evidence of persistent genetic damage in the airways as well as in the etiological role of tobacco in genetic alterations. Such agents include retinoids, carotenoids and N-acetylcysteine. However, there have been conflicting results on the beneficial effects of these agents.\(^1\)\(^2\)\(^,\)\(^11\)

Passive smoking also increases the risk of developing lung cancer. Former smokers present a progressive risk reduction after a minimum of five to twenty years without smoking.\(^2\)\(^,\)\(^10\) Although most lung cancer cases are smoking-related, only a minority of heavy smokers develop lung cancer. This phenomenon is related to the fact that genetic factors can affect individual susceptibility.\(^12\)\(^,\)\(^13\) The incidence of different histological types in lung cancer is influenced by smoking. Squamous cell carcinoma, and SCLC in particular, present a strong, direct correlation with tobacco use that is stronger than that found for adenocarcinoma, which is clearly more common among women.\(^13\)\(^-\)\(^14\)

### SMOKING AS A PROGNOSTIC FACTOR

There are various clinical, anatomopathological, radiological and laboratory testing factors related to lung cancer clinical presentation, response to treatment and survival. Such factors include histological type, weight loss, performance status, tumor staging and tumor markers.\(^15\)\(^,\)\(^16\) Despite the variety of prognostic factors, their complexity and the cost of testing hinder their wider use in research and in clinical practice. Prognostic studies have allowed the choice of more appropriate practices, in accordance with the tolerance to treatment, chance of response and probable survival.\(^16\)\(^,\)\(^16\)

Since the 1960s, there has been interest in describing clinical, radiological and laboratory testing factors that would permit better prognostic analysis.\(^15\)\(^-\)\(^16\) In a study involving 1,155 lung cancer patients, in which the principal variables analyzed were age, gender, histology and staging, smoking was found to be a significant and independent predictor of poor survival.\(^17\)

Smoking has been correlated with other factors that could contribute to poor survival in lung cancer: lower socioeconomic level,\(^18\) worse nutritional state,\(^19\) accompanying diseases,\(^20\) immunosuppression,\(^21\) and mutations that promote carcinogenesis.\(^22\) Of these, the presence of concomitant diseases seems to be the most important factor since smoking has been correlated with various diseases other than lung cancer.

Smoking patients with lung cancer can die from various smoking-related diseases. Although it is commonly thought that nearly all lung cancer patients die from lung cancer, this is not true. From 20% to 40% of patients with nonmetastatic lung cancer die without any evidence of progression of the disease.\(^23\) Therefore, the correlation between smoking and decreased survival, at least among patients with nonmetastatic lung cancer, might be attributable to smoking-related comorbidities.

Smoking patients can present decreased survival because they do not receive full treatment, possibly because smoking is associated with a lower socioeconomic level, with the deterioration of pulmonary function or with comorbidities, which causes these patients to be excluded from a more radical treatment.\(^24\)

### IMPACT OF SMOKING ON HEALTH AND ON WOMEN

The World Health Organization estimates that smoking accounts for five million deaths per year worldwide.\(^25\) Exposure to tobacco smoke through inhalation of large doses causes cancer as well as cardiovascular and respiratory diseases. Lung cancer rates among moderate smokers (one to nine cigarettes a day) are, on average, six times higher than among nonsmokers, which indicates smoking is a great risk factor even when exposure is low and that there are no safe levels of exposure. Even smokers who do not inhale (cigar and pipe smokers) present a high lung cancer risk, approximately tenfold higher than that of nonsmokers.\(^26\)

Women appear to be more susceptible than men to tobacco carcinogens. Even when women smoke the same amount of cigarettes as men they present higher rates of lung cancer.\(^14\) Lung cancer-related mortality among women is increasing at a faster rate than that seen among men. From 1979 to 1999, worldwide mortality from this type of cancer increased by 57% among men and by 122% among women.\(^25\)
LOW-TAR CIGARETTES, PASSIVE SMOKING AND ENVIRONMENTAL TOBACCO SMOKE

After it was demonstrated that cigarettes could cause cancer, cigarette industries started adding filters to them, developing low-tar and low-nicotine cigarettes. The ventilation devices of the filters are holes or perforations that serve to dilute the smoke inhaled by the smoker with entrained air, thereby reducing the concentrations of tar and nicotine, as well as reducing carbon dioxide emissions.\(^{27}\)

One way for the smoker to compensate for the reduced nicotine emission caused by the dilution of the smoke with the air entrained through the holes in the cigarette filters is by inhaling more deeply to increase the volume of smoke inhaled. When switching from a cigarette brand with regular nicotine yield to another with a lower yield, the smoker immediately begins a process of compensatory smoking through changing the way they smoke, and they obtain the necessary nicotine to satisfy their dependence by increasing puff volume and inhaling the smoke more deeply into the lungs.\(^{27}\)

A cohort study\(^{28}\) analyzed the risk of developing lung cancer among smokers of medium-tar, low-tar and very low-tar filter cigarettes who were loyal smokers of the same brand for over ten years in comparison with former smokers and nonsmokers. Regardless of the tar yield of the brand smoked, smokers presented a higher lung cancer risk than did former smokers and nonsmokers. Lung cancer risk was similar among smokers of medium-tar, low-tar and very low-tar cigarettes, which is consistent with the compensatory smoking phenomenon.\(^{28}\)

The burning of tobacco derivatives results in the formation of two kinds of smoke streams: mainstream smoke (which the smoker pulls through the mouthpiece when he inhales or puffs) and sidestream smoke (smoke generated during the interval between puffs that drifts freely from the burning end of a cigarette and enters the air directly into the environment). The term environmental tobacco smoke refers to the tobacco smoke released into the environment that people inhale involuntarily. Studies on passive smoking reveal that lung cancer risk is 30% higher among nonsmokers chronically exposed to environmental tobacco smoke than among nonsmokers who have not been exposed.\(^{29}\)

SMOKING AND MOLECULAR CHANGES IN LUNG CANCER

Over 4,000 chemicals and as many as 60 carcinogenic substances have been identified in tobacco smoke. The main classes of carcinogenic compounds found in tobacco smoke include polycyclic hydrocarbons (such as benzopyrene), nitrosamines and aromatic amines. These substances can promote damage to the deoxyribonucleic acid (DNA) by activating procarcinogenic compounds (phase I enzymes), a situation that is counterbalanced by the capacity of detoxifying carcinogens (phase II enzymes).\(^{12}\) The capacity to repair DNA has been shown to be significantly lower in patients with lung cancer.\(^{12}\)

Approximately 50 tumor suppressor genes and over 100 oncogenes have been described, and, since they intimately participate in the regulation of cell growth and division, lung cancer can be considered to be cell cycle dependent.\(^{32-34}\) The cell cycle model consists of an S phase (DNA synthesis) and an M phase (mitosis), separated by 2 additional phases (G1 and G2).\(^{33}\)

Substances present in tobacco can act as carcinogens or, more typically, as procarcinogens that need phase I enzymes (encoded by cytochrome P450 - CYP genes) in order to activate their carcinogenic effects. These carcinogens might link to DNA, thereby inducing mutations and carcinogenesis.\(^{12,34}\) The CYP family is divided into ten subfamilies (CYP1-10), and the CYP1-4 subfamilies are primarily involved in the metabolism of drugs.\(^{12,34}\) Phase I enzymes metabolically transform procarcinogens into intermediates that are toxic to the cells and can link to the DNA or be transformed by phase II enzymes (through conjugation) into hydroxysoluble intermediates that are excreted by the cells. Individuals who present efficient phase I metabolism and impaired phase II metabolism accumulate intermediates that are toxic to the cells, thus increasing lung cancer risk. The enzymes involved in the activation and conjugation of the
constituents of tobacco are cytochrome P450, phase I enzymes (CYP1A1 and CYP2E1) and phase II enzymes. Phase II enzymes include N-acetyltransferase and glutathione S-transferase, which consists of five families: actinin, sigma factor, heavy chain disease, mesons and theta. Various studies have demonstrated the correlation between smoking and lung cancer-related molecular changes.(15,22,34-42) These principal changes and their frequencies in NSCLC and SCLC are summarized in Charts 1 and 2.

TUMOR SUPPRESSOR GENES

There are two classes of oncogenes: dominant and recessive (or tumor suppressor genes). Dominant, as opposed to recessive, oncogenes are easily identified because they have a dominant genetic effect of converting a normal cell into a malignant cell. To that end, the mutation needs only to affect one of the two dominant oncogene alleles, whereas carcinogenesis occurs in recessive oncogenes only when the mutation affects the pair of alleles. Molecular mechanisms of oncogene activation include amplification, mutation at a specific point in time and translocation, as well as overexpression of the protein or of the level of gene transcription.(2,15)

The first confirmation of the existence of tumor suppressor genes was the identification of the retinoblastoma (Rb) gene. Flaws in the Rb gene are almost universal in SCLC, but they are observed in only 30% of NSCLCs. The Rb gene, located on the long arm of chromosome 13 (13q), encodes a regulatory nuclear phosphoprotein from the cell division. The role of altered Rb and p53 expression in NSCLC has been studied. Abnormalities in the p53 and Rb pathways (increased expression of cyclin D1 and mutations in p53) have been found to indicate a worse prognosis. However, since there is a great complexity and interaction among different proteins, such results need to be further evaluated.(35)

The tumor suppressor gene p53 is the gene that most commonly becomes mutated in lung cancer. The p53 gene is located on the short arm of chromosome 17 (17p), and its mutation occurs almost exclusively in codon 157 when related to lung cancer and smoking. Mutations of the p53 gene promote guanine-to-thymine (G-to-T) transversions. The normal p53 protein can induce p21, resulting in dephosphorylation of Rb and inhibition of the cell cycle. The p53 gene regulates cell growth in the G1-S interface of the cell cycle and plays an important role in inducing the apoptosis of cells with damaged DNA.(36-37)

The p53 protein is important in the apoptosis of cells with damaged DNA, and mutations in the p53 gene usually reflect exposure to environmental carcinogens (for example, smoking and lung cancer). In its wild form, p53 protein regulates cell growth and mutations in the p53 gene can inhibit or abolish the production of p53 protein. In its mutant form, p53 protein generally lives longer than does the wild type, resulting in high p53 protein levels in

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Chart 1 - Principal molecular markers found in lung cancer

<table>
<thead>
<tr>
<th>Tumor suppressor genes</th>
<th>p53, Rb, p16, p21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proto-oncogenes</td>
<td>K-ras, c-myc, c-erb-1 and 2, HGF, HER-2</td>
</tr>
<tr>
<td>Telomerase</td>
<td>hTERT</td>
</tr>
<tr>
<td>Hypermethylation and growth factors</td>
<td>GRP/BN, TGF-b, FDGF, PTHrP, IGF-1 and II</td>
</tr>
<tr>
<td>Apoptosis and angiogenesis</td>
<td>Bel-2, VEGF</td>
</tr>
<tr>
<td>Gene amplification</td>
<td>HER-2</td>
</tr>
</tbody>
</table>

Source: adapted from references 2, 15 and 22

Chart 2 - Principal differences in the frequencies of molecular markers in lung cancer

<table>
<thead>
<tr>
<th>Molecular marker</th>
<th>NSCLC (%)</th>
<th>SCLC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>40-60</td>
<td>40-70</td>
</tr>
<tr>
<td>Rb</td>
<td>30</td>
<td>~100</td>
</tr>
<tr>
<td>p16</td>
<td>10-40</td>
<td>&lt;1</td>
</tr>
<tr>
<td>K-ras</td>
<td>15-20</td>
<td>&lt;1</td>
</tr>
<tr>
<td>c-myc</td>
<td>10</td>
<td>80-90</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>12-25</td>
<td>75-80</td>
</tr>
<tr>
<td>c-erB-2</td>
<td>25</td>
<td>&lt;5</td>
</tr>
<tr>
<td>FHIT</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Telomerase</td>
<td>80-85</td>
<td>~100</td>
</tr>
<tr>
<td>GRP/BN</td>
<td>Raro</td>
<td>20-60</td>
</tr>
</tbody>
</table>

NSCLC: non-small cell lung carcinomas; SCLC: small cell lung carcinomas.
Source: adapted from references 2, 15 and 22
Molecular markers in lung cancer: prognostic role and relationship to smoking

Malignant cells. The p53 gene encodes a nuclear transcription factor that links to the p21 promoter, inducing its expression and inhibiting the progression of the cell cycle in the G1/S stage. In contrast, mutant p53 protein is not capable of activating p21. Therefore, the p53 gene is considered the “genome guardian” and protects respiratory system cells from environmental carcinogens.\(^{36-37}\) In lung cancer, mutations in the p53 gene have been shown to correlate strongly with smoking. Abnormal p53 gene expression has been observed in 40% to 70% of all SCLCs and in 40% to 60% of all NSCLCs, the squamous cell histological type being more common than the adenocarcinoma histological type.\(^{36-40}\)

In mixed pulmonary tumors, abnormalities in p53 status appear to be unrelated to prognosis but seem to worsen prognosis in patients with NSCLC.\(^{37}\) An apoptosis-promoting gene known as bax can be induced by p53.\(^{15}\) The obvious importance of the mutations in p53 in the pathogenesis of lung cancer notwithstanding, it remains unclear whether these changes affect prognosis since the results are still conflicting.\(^{15}\) A meta-analysis of p53 has revealed that it has no prognostic value in SCLC. However, it is a factor for poor prognosis in all stages of NSCLC.\(^{37}\)

Replication of DNA is inhibited by p21, the expression of which increases in parallel with increased expression of p53. The expression of p21 in lung cancer is increased by 65% to 75% in NSCLC, especially in well-differentiated tumors.\(^{2,15,41}\) The results obtained regarding the importance of p21 as a prognostic factor are also conflicting.\(^{15,41}\) The pathway of the tumor suppressor protein p16 undergoes mutation in various types of cancer. It is detected in 10% to 40% of NSCLCs and in less than 1% of SCLCs.\(^{2,15,42}\) Hypermethylation of p16 (INK4a) is an unfavorable prognostic factor, mainly in adenocarcinomas. In NSCLCs accompanied by p53 and p16 expression, the postresection prognosis has been shown to be worsened, principally in the initial stages.\(^{42}\)

**PROTO-ONCOGENES AND GROWTH STIMULATORS**

There are three proto-oncogenes in the ras family: K-ras, N-ras and H-ras.\(^{38,43-44}\) These genes encode binding proteins of the guanosine triphosphate group, known as p21ras, which are easily correlated with and present a structural similarity to the G protein.\(^{15,41,43-44}\) These proteins are located on the internal side of the cell membrane and participate in signal transduction. Activation of K-ras by mutation at a specific point occurs in 50% of the pulmonary adenocarcinomas, which can be determined by polymerase chain reaction of cells collected through bronchoalveolar lavage of patients with lung cancer.\(^{12,15,43-44}\) This mutation results in a single modification in the amino acid of the protein, leading to a significant decrease in the activity of the intrinsic guanosine triphosphate, and the protein remains in an active binding state with the guanosine triphosphate, which hinders its release. Once acquired, these mutations remain stable, both in primary and metastatic tumors. Although still controversial, mutations at codon 12 of the K-ras oncogene can worsen the prognosis for patients with this malignancy, especially when multimodal therapy is performed.\(^{2,15,43,45}\)

Most ras mutations are defects in the activity of the guanosine triphosphate, which is the active form of K-ras. When such a mutation is combined with hypermethylation of the ras association domain family 1A promoter, the consequent synergic effect is a negative prognostic factor.\(^{44,46}\) Mutations in K-ras occur in 20% to 50% of adenocarcinomas (15% to 20% of all NSCLCs), but they rarely occur in cases of SCLC.\(^{2,15,46}\)

Mutations in K-ras oncogenes have been strongly correlated with smoking, and G-to-T transversions are usually caused by polycyclic hydrocarbons and nitrosamines.\(^{47}\) In adenocarcinomas, including completely resected NSCLCs, a K-ras mutation is usually a negative prognostic factor.\(^{38}\) Mutations in other members of the ras family (N-ras and H-ras) occur less frequently in NSCLCs and are rare in lung cancer. The protein product of the ras gene (p21ras) has also been shown to be a significant prognostic factor in cases of NSCLC.\(^{43-47}\) Mutations in K-ras genes have been detected in bronchial biopsies of smoking individuals without lung cancer, as well as in sputum collected before the diagnosis of malignancy, acting as a premalignancy marker.\(^{43-47}\)

The myc family of proto-oncogenes includes nuclear proteins that can link to DNA and act in the regulation of the transcription. The myc family has 3 members: c-myc, N-myc and L-myc. The c-myc proto-oncogene is amplified principally in SCLCs (80% to 90%) and less frequently in NSCLCs (approximately 10%), and its amplification has been correlated with a more aggressive course in cases.
of SCLC. Expression of this protein has been shown to be greater in lung tumors than in samples of non-neoplastic pulmonary tissue. Expression of N-myc genes has been correlated with worse response to chemotherapy. The amplification of myc genes also occurs in NSCLCs. However, to date, this has not been shown to have clinical significance.\(^{(48)}\)

Some oncogenes exert their effects through the hyperproduction of normal encoding proteins, suggesting a regulatory defect in the transcription or amplification of the gene. Genes and protein products that typically act in this way are the c-erbB-1 and c-erbB-2 proto-oncogenes, both of which are tyrosine-kinase receptors. The c-erbB-1 proto-oncogene is most frequently found in NSCLCs, especially in squamous cell carcinomas (65% to 90%), although it has not been correlated with prognosis. The c-erbB-2 proto-oncogene is most frequently found in adenocarcinomas.\(^{(49)}\)

The c-erbB-1 proto-oncogene, when linked to the membrane, encodes a tyrosine-kinase growth receptor that is the receptor for the epidermal growth factor. The use of the c-erB-1 proto-oncogene as a prognostic factor in lung cancer is controversial. The c-erbB-2 proto-oncogene is structurally related to the c-erB-1 proto-oncogene, and its encoding protein, known as HER2, is also expressed in epithelial cells of the airways of the normal lung. In many adenocarcinomas, HER2, which is a transmembrane protein with tyrosine-kinase activity, is produced in conjunction with the receptor for the epidermal growth factor, and serum levels of HER2 have been correlated with tumor burden. Higher HER2 levels (>22 U/ml) are found in stage IIIB and IV NSCLC tumors (especially in adenocarcinomas), promoting worse prognosis.\(^{(2,15,49)}\) Increased c-erB-2 expression is rare in SCLCs (<5%) but can be found in 25% of NSCLCs.\(^{(2,15,49)}\)

Mutation of K-ras, when accompanied by other molecular changes, has greater value than does K-ras in isolation.\(^{(15)}\) The combination of K-ras mutations and increased expression of p185 worsens the prognosis for patients with adenocarcinoma, as well as for patients with resected NSCLC when all three markers (p53, K-ras and c-erbB-2) are present.\(^{(55)}\)

Another membrane tyrosine-kinase is the hepatocyte growth factor. The hepatocyte growth factor stimulates the proliferation of epithelial cells, stimulates mitosis of the human bronchial epithelium, as well as of type II alveolar cells, and is expressed mainly in NSCLCs, resulting in a worse prognosis. The hepatocyte growth factor also mediates angiogenesis, cell motility and invasion. It is expressed in normal bronchial epithelial cells and in most cases of NSCLC but is rarely produced in SCLCs.\(^{(50)}\)

**TELOMERASE ACTIVATION**

Telomerase activation is a potential lung cancer marker. Although telomerase activity has been found to be very low or absent in non-neoplastic tissue, it is present in most cancer cells, resulting in cell “immortality”.\(^{(51-52)}\) The integrity of the telomeres is fundamental for the maintenance of the chromosomal stability and for preventing the fusion of chromosomes and translocations.\(^{(51-52)}\)

Telomeres are protective sequences that constitute the ends of the chromosomes and are involved in practically all types of cancer in men. Expression of the three main components of human telomerase (ribonucleic acid component, telomerase-related protein and catalytic subunit) is increased in lung cancer. High levels of telomerase activity are seen in nearly all SCLCs and in 80% to 85% of all NSCLCs. Highly elevated telomerase activity has been correlated with advanced stages of NSCLC\(^{(51-52)}\) but can be found in in situ carcinoma, which implies the involvement of initial stages of lung cancer. Telomerase activity has principally been detected in samples of abnormal bronchial epithelium: hyperplasia (in 71%); metaplasia (in 80%); and in situ carcinoma (in 100%).\(^{(52)}\) The acquisition of telomerase activity represents a major event in tumorigenesis since it is a significant molecular marker of lung cancer.\(^{(51-52)}\)

**HYPERMETHYLATION AND GROWTH FACTORS**

In NSCLC, the hypermethylation of the p16 promoter contributes to the decreased levels of gene transcription seen in the initial stages of pulmonary carcinogenesis and has been found to correlate strongly with smoking.\(^{(51-53)}\) Stimuli of autocrine and paracrine growth are present in lung cancer as a consequence of the expression of growth factors, regulator peptides and receptors by malignant cells or by normal adjacent cells. However, most such factors are proto-oncogene products.\(^{(54)}\) Chief among
these factors is bombesin (gastrin releasing peptide), which is expressed in 20% to 60% of SCLCs and only rarely in NSCLCs.\(^{53}\)

The transforming growth factor-beta cytokine is a promoter of lung inflammation and can link to integrins that are expressed by tumor cells. This growth factor can inhibit the G1/S phase, as well as producing p21 and expressing c-myc.\(^{41,54}\) The transforming growth factor-beta-1 can affect tumor angiogenesis, play an important role in tumor progression in NSCLC and act as an independent predictor of survival in adenocarcinoma. Other implicated growth factors include the platelet-derived growth factor, parathyroid hormone-related protein and insulin-like growth factor.\(^{2,15}\)

Expression of Ki-67 is a prognostic marker in patients with NSCLC (principally adenocarcinoma) who have undergone surgery and has been correlated with smoking.\(^{55}\) Patients with stage I NSCLC presenting hypermethylation in the DAP kinase gene promoter present poorer five-year survival rates than do those who present no such hypermethylation. In SCLC, regional hypermethylation has been found in chromosome 3p, although the specific promoter remains unknown.\(^{2,15,54-55}\)

APOPTOSIS AND ANGIOGENESIS

The anti-apoptotic proto-oncogene Bcl-2 plays a role in apoptosis. Expression of Bcl-2 is higher in SCLCs (75% to 95% of cases) than in NSCLCs (25% of squamous cell carcinomas and 12% of adenocarcinomas).\(^{38,56}\) Patients with SCLC and Bcl-2 expression present improved survival, although the role played by Bcl-2 expression in NSCLC is controversial. Typical and atypical carcinoid tumors occur concomitantly with low levels of Bcl-2.\(^{38,56}\)

Tumors require angiogenic factors early in their pathogenesis. This process is a complex phenomenon and involves various inducers and inhibitors, resulting in endothelial cell proliferation and migration. The vascular endothelial growth factor (VEGF) and the fibroblast growth factor are important angiogenesis inducers.\(^{55}\)

Expression of VEGF in increased in metastatic lung cancer, worsening the prognosis in cases of NSCLC. In squamous cell carcinoma, the VEGF receptor (Flt-1) is constantly expressed, suggesting an autocrine role of VEGF in Flt-1, and the mutant p53 acts synergistically with hypoxia to express VEGF. VEGF increases microvascular permeability and stimulates endothelial cell growth. Increased expression of VEGF, p53, Rb and Bcl-2 can affect resistance to chemotherapy.\(^{57}\) Expression of VEGF is a prognostic indicator in patients with lung cancer, participating in tumor progression through two mechanisms: as a growth factor for tumor cells and as a mitotic factor for vascular endothelial cells.\(^{57}\)

Smoking causes the loss of fragile histidine triad (FHIT) gene function, and carcinogens present in tobacco smoke cause a deletion in the FHIT allele. Continuous exposure causes deletion in the second allele, leading to the loss of FHIT expression. This loss of expression is more common in smokers than in nonsmokers (75% vs. 39%) and has been correlated with worse prognosis (mainly in cases of NSCLC). Altered FHIT expression is found in 80% of all SCLCs and in 40% of all NSCLC. Fibroblast growth factor is expressed in 70% of all NSCLCs, but its importance in prognosis remains undefined.\(^{2,15,57}\)

GENETIC ABNORMALITIES - AMPLIFICATIONS, ALLELE DELETIONS, MUTATIONS

Gene amplifications, allele deletions and punctual mutations are commonly described in lung cancer. Gene amplifications, such as that of HER-2/neu, are more commonly associated with advanced stages and worse prognosis. Punctual mutations are more frequent in advanced stages of the disease and are rarely observed in premalignant lesions or in the normal bronchial epithelium of smokers. Allele deletion has been demonstrated in invasive tumors as well as in preneoplastic lesions. These deletions result in the loss of tumor suppressor genes and favor the development of neoplasia. Some chromosomal regions, such as the short arms of chromosomes 3, 9 and 17, are commonly deficient in lung cancer.\(^{2,15,58-60}\)

CHROMOSOMIC ABNORMALITIES - ANEUPLOIDIES, DELETIONS

Numeric alterations such as losses or gains of chromosomes have also been described in lung cancer and in premalignant lesions. Numeric chromosomal changes or aneuploidies have been detected with flow cytometry. Notable among the various methods for the
smokers and therefore at high risk. Of this factor, mainly in patients who are heavy it is important to emphasize the need for prevention abnormalities in lung cancer are smoking-related, J Bras Pneumol. 2005; 32(1):56-65

CONCLUSIONS

Despite new therapies, survival in lung cancer remains poor. Molecular markers can be important tools for understanding pulmonary carcinogenesis, making early diagnoses, determining prognoses and identifying new treatments for patients with lung cancer. Since the great majority of these abnormalities in lung cancer are smoking-related, it is important to emphasize the need for prevention of this factor, mainly in patients who are heavy smokers and therefore at high risk.

REFERENCES