REVIEW

Lung cancer and smoking: molecular aspects

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Abstract

Lung cancer (LC) is characterized as one of the most common and lethal types of cancers worldwide, with approximately 230.000 new cases each year in the US and 160.000 deaths are estimated for 2012. In Brazil, the outlook is also bleak, with 27,320 new cases expected in 2012, according to the Brazilian National Cancer Institute (INCA). LC is classified into two major histological types: small cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). In addition to sizable mortality and incidence, LC has low 5-year survival rates when compared to other types of common cancers such as breast and prostate, even with recent diagnostic and therapeutic advances. For the survival rate of patients with LC to increase, a greater understanding of the molecular events that lead to the emergence of this malignancy is necessary in order to identify genetic markers involved in tumor progression, and thus enable early detection and to develop new specific therapeutic strategies, allowing for a more individualized treatment in patients with LC. Different situations are classified as risk factors for the development of LC, but unquestionably, the most responsible risk factor for the high incidence of LC in the world population by far is smoking.

Keywords: carcinoma, lung neoplasms, molecular biology, non-small-cell lung, smoking.

INTRODUCTION

Lung cancer (LC) represents a major health challenge worldwide. A total of 160.000 deaths from lung cancer (87.000 men and 73.000 women) were estimated in the United States in 2012, accounting for approximately 29% of all cancer deaths. However, LC is the second most frequent cancer in men (after prostate cancer) and women (after breast cancer), totaling 15% of the new cases of cancer, with 230.000 cases expected for 2012[1]. In Brazil, there are an estimated 27.320 new cases of lung, tracheal and bronchial cancer in 2012[2]. LC occurs primarily in elderly individuals; around two out of three patients diagnosed with LC are aged 65 and older, while less than 3% of all cases occur in people under 45 years[1].

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In the early 20th century, LC was much less frequent than other types of cancers, but with the manufacture and sale of cigarettes on a large scale, the number of cases increased dramatically. Prevention is the most effective method in an attempt to reduce mortality from LC, and a crucial point is to prevent new people from starting to smoke, and for those who smoke, the implementation of viable methods to stop smoking. These combined strategies have had a significant impact in the United States in the second half of the 20th century, in which there has been a decrease in the prevalence of adult smokers from 42.4% in 1965 to 25.5% in 1990. Similarly, there was a reduction in deaths from LC among men in this period[3]. However, since 1990 there have not been significant decreases in the prevalence of smoking in the United States.

In this respect, the aim of this literature review was to elucidate the molecular mechanisms involved in the pathogenesis of malignant LC, which constitutes the leading cause of death from cancer among men and women, and their direct relationship with smoking.

Lung cancer: NSCLC

LC is classified into two major histological types: small cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). If the tumor has characteristics of both histological types, then it is called mixed small cell/large cell cancer, which is highly uncommon[1]. NSCLC is the most prevalent, representing approximately 80 to 90% of LC cases and is histologically subdivided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma[4]. Adenocarcinomas are the most common,

representing about 40% of LC cases[4]. Usually, they are found in the periphery of the lung and can quickly metastasize to the liver, adrenal glands, bones or brain[6]. However, patients with any type of adenocarcinoma tend to have a better prognosis compared to individuals who are diagnosed with other subtypes of LC[1]. Adenocarcinomas, according to a recent reclassification can be divided into: adenocarcinoma *in situ* (AIS), minimally invasive adenocarcinoma (MIA), lepidic-predominant adenocarcinoma, predominantly invasive adenocarcinoma with some nonmucinous lepidic components and invasive mucinous adenocarcinoma[5], characterized typically by a multifocal inflammatory pattern[6].

Regarding squamous cell carcinoma, this represents about 25 to 30% of all LC cases and is also commonly associated with a history of smoking[1,4]. Squamous cell carcinoma tends to be found in the central region of the lungs, proximal to the bronchi, often resulting in endobronchial obstruction and hemoptysis[6]. Large cell carcinoma may appear in any part of the lung, and in addition to growing and spreading rapidly, it hampers the success of treatment. This subtype represents approximately 10 to 15% of LC cases but their frequency has declined in part due to improved diagnostic techniques, which is now categorized into adenocarcinoma or squamous cell carcinoma[1].

Currently, more people die from LC than from cancers of the colon, breast and prostate combined[1] and these malignancies have demonstrated significant increases in 5-year survival rates, representing, respectively, 99%, 88% and 64%, whereas this rate for LC remains relatively stable at 15%. There are some possible explanations for this disparity between the survival of patients with LC and other common tumors, such as delayed detection and heterogeneous histology, which makes LC difficult to accurately diagnosis and therefore direct treatment course. Over 75% of new LC cases are diagnosed in patients with regional or distant metastases[7]. Histologically, carcinomas of the prostate, breast and colon are uniformly adenocarcinomas and treatment is determined by clinical stage and molecular analyses[8-9]. LC has a great heterogeneous histology and, until recently, subtypes of NSCLC were treated similarly. The most effective treatment currently available for NSCLC is still surgical resection, however, more than 70% of patients have advanced disease with nodal and/or visceral metastases at diagnosis, which precludes resection[10]. Thus, as the survival rate of patients with LC increases, a greater understanding of the molecular events that lead to the emergence of LC is necessary in order to identify genetic markers involved in tumor progression, and thus enable early detection and to develop new specific therapeutic strategies, allowing for a more individualized treatment in patients with LC.

LC is characterized by the sequential accumulation of multiple genetic and morphological changes that lead to the evasion of apoptosis and alterations in DNA repair and stability, tissue invasion, metastasis and angiogenesis. Several genetic abnormalities occur during the induction of LC, including: loss of heterozygosity; microsatellite alterations; RAS oncogene mutations; MYC amplification; Bcl2 expression; mutations in p53, RB, p16 and FHIT tumor suppressor genes, and; the expression of telomerase activity, among others[11-12]. Between 45-75% of NSCLCs have mutation in p53[13], a tumor suppressor gene considered the "guardian of the genome". Since the p53 gene is mutated or deleted, the cells become susceptible to DNA damage and uncontrolled cell growth. Mutations in p53 are configured as one of the most important tools for early detection and diagnosis of LC, and the most common mutation in this gene is a GC to TA transversion, with strong correlation between the frequency of this mutation and exposure to tobacco[10]. Approximately 70-80% of the somatic mutations of p53 found in NSCLC prolong the half-life of p53 by promoting increased protein levels detectable by immunohistochemistry, unlike wild-type p53, which is not routinely detectable by this technique[14]. A clinical meta-analysis suggested that alterations of p53 (molecularly detected by immunohistochemistry) may represent a significant marker of worse prognosis in patients with lung adenocarcinoma[15].

The p16-cyclin D1/Cdk4-Rb pathway has also been evaluated in LC, and this is fundamental in controlling the transition from G1 phase into the S phase of the cell cycle, with abnormalities in its components often found in individuals with NSCLC. Between 30-70% of NSCLC tumors have some type of mutation in p16, including homozygous deletion, point mutations or epigenetic alterations[11], while 15-30% had alterations to the RB gene, another key component of this pathway in which the loss of function may result from deletions, mutations or abnormal splicing[16-17]. The absence of RB protein expression was associated with poor prognosis in one study[18], but not confirmed in others[19-20]. Another group of genes that has been studied in LC are the tumor suppressor genes, and a component of that group, the FHIT gene, has 40% of the aberrant transcripts in specimens of NSCLC and absence of protein expression in approximately 50% of cases of LC[21-22]. The properties of the FHIT tumor suppressor became evident after cell transfection studies in which one copy of wild-type strain FHIT was transfected into lung cell lines. In these studies, we observed that tumorigenicity was suppressed and apoptosis was induced,[23-24] suggesting that FHIT overexpression could serve as a future therapeutic approach.

The proto-oncogene c-erbB-1, which encodes the epidermal growth factor receptor (EGFR), is also overexpressed in LC in approximately 13% of cases of NSCLCs[25]. EGFR regulates epithelial proliferation and differentiation[26] and the expression of this protein is a risk factor to the condition of worse prognosis in patients with NSCLC.[27] The proto-oncogene c-erbB-2, also called

HER2/neu, is overexpressed in approximately 30% of NSCLCs, especially in adenocarcinomas[28] and is associated with shorter survival and intrinsic resistance to chemotherapy and radiotherapy[29]. The use of humanized anti-HER2 receptor monoclonal antibodies (trastuzumab, trade name Herceptin) was tested in breast cancer with promising results and has been assessed in studies of NSCLC[30]. Moreover, molecule inhibitors of tyrosine kinase (gefinitib and erlotinib), have been evaluated in the treatment of patients that express EGFR, and gefinitib has already been approved for use by the US Food and Drug Administration (FDA) for NSCLC patients. KRAS, a member of the RAS oncogene family, encodes a protein which is involved in signal transduction, and mutations in this gene, usually in codons 12 and 13, have been reported in more than 30% of lung adenocarcinomas and in 15-20% of all cases of NSCLC. Such alterations are often found in smokers[31-32] and a meta-analysis involving 891 cases of NSCLC suggested that the presence of KRAS mutations is associated with worse prognosis[33]. Finally, the BCL-2 protein is often expressed in NSCLCs, especially squamous cell carcinomas, based on studies using the technique of immunohistochemistry[34]. A higher response rate to chemotherapy was observed for BCL-2-positive tumors compared to BCL-2-negative tumors, suggesting that the expression of BCL-2 may reflect a greater susceptibility to chemotherapy treatment[35].

NSCLC Staging

NSCLC presents staging following the American Joint Committee on Cancer (AJCC) TNM staging system (Tables 1-3 and 4), which considers the size and site of the primary tumor (T), lymph node involvement (N), and the presence of distant metastasis (M). Approximately 75% of all NSCLC cases are diagnosed with regional or metastatic disease, whereas less than 20% are considered local at diagnosis[4]. The 5-year survival rate is reduced by 56% in patients with stage I to approximately 2% in stage IV, the most advanced of the disease[1].

Inaccurate staging of LC negatively affects the outcome of therapy, while a precise staging of the disease provides the determination of the extent of LC correctly, an effective prognostic stratification, as well as selecting the most appropriate treatment. When the disease stage is underestimated, the patient may not receive the benefits of certain systemic therapies, and when the opposite occurs and the stage is overestimated, the patient may be denied surgical resection or other curative processes[36]. Patients with stage I NSCLC undergo resection of the primary tumor (without adjuvant therapy) as standard treatment[37] and survival for patients in this stage varies between 40 and 70%, with the main reason for treatment failure is due to distant recurrences[38]. These data suggest that a significant number of patients diagnosed with stage I disease may actually have underestimated staging. If properly diagnosed, these

Table 1. Definition of the tumor (T) stage of the TNM staging system.

TNM Staging	
Tumor	Description
TX	Primary tumor cannot be assessed or is detected by the presence of malignant cells in sputum or bronchial washings, but the tumor cannot be visualized by imaging or bronchoscopy
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor 3 cm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus
T1a	Tumor size, 2 cm or less
T1b	Tumor measuring between 2 and 3 cm
T2	Tumor with any of these characteristics of size or extent: more than 3 cm in greatest dimension; involvement of the main bronchus, 2 cm or more distal to the carina, and invades the visceral pleura. Associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung
T2a	Tumor measuring between 3 and 5 cm
T2b	Tumor measuring between 5 and 7 cm
T3	Tumor size greater than 7 cm that invades directly: chest wall, diaphragm, mediastinal pleura, parietal pericardium, or tumor in the main bronchus less than 2 cm distal to the carina, but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung. Separate nodules in the same lobe
T4	Tumor of any size that invades: mediastinum, heart, great vessels, trachea, esophagus, vertebral body, carina, separate tumor nodules in a different ipsilateral lobe

Source: AJCC Cancer Staging Handbook (7 ed.).

Table 2. Definition of the regional lymph node (N) stage of the TNM staging system.

TNM Staging	
Regional lymph nodes	Description
NX	Regional lymph nodes cannot be assessed
N0	No metastases in regional lymph nodes
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes including involvement by direct extension of the primary tumor
N2	Metastasis in ipsilateral mediastinal, contralateral hilar, contralateral or ipsilateral scalene, or supraclavicular lymph nodes

Source: AJCC Cancer Staging Handbook (7 ed.).

Table 3. Definition of distant metastasis (M) stage of the TNM staging system.

TNM Staging	
Distant metastasis	Description
MX	Distant metastasis cannot be assessed
M0	There is no distant metastasis.
M1a	Separate tumor nodules in a contralateral lobe: pleural nodules or malignant pleural (or pericardial) effusion
M1b	Presence of distant metastasis

Source: AJCC Cancer Staging Handbook (7 ed.).

Table 4. Lung cancer staging.

	Staging		
Stage	Tumor	Node	Metastasis
Occult carcinoma	TX	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T2b	N0	M0
	T1a,b	N1	
	T2a	N1	
Stage IIB	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T1a,b	N2	M0
	T2a,b	N2	M0
	T3	N1	M0
	T3	N2	M0
	T4	N0	M0
	T4	N1	M0
Stage IIIB	Any T	N3	M0
	T4	N2	M0
Stage IV	Any T	Any N	M1

Source: AJCC Cancer Staging Handbook (7 ed.).

patients could benefit from the effects of adjuvant therapy in addition to resection, resulting in a possible increase in survival rates. Thus, the importance of accurate staging is clear in treating and obtaining therapeutic success.

Molecular biological staging, namely, the evaluation of primary tumors with the use of molecular techniques, can theoretically improve the prognostic stratification of patients with NSCLC, enabling to predict which patients are most likely to experience disease recurrence after surgical resection. Moreover, the profile of the primary tumor can be used in the analysis of sensitivity to possible adjuvant therapies selected. However, the sole use of oncogenic markers is not effective in predicting the prognosis of the patient, since a "reduced" percentage of tumors is present due to the frequency of aberrant expression of many of the markers. For example, the overexpression of p53 and EGFR are observed in approximately 43% and 52% of NSCLCs, respectively. Thus, the use of a panel of molecular markers is believed to be ideal. Molecular biological staging also offers an opportunity to individualize chemotherapy regimens according to the molecular profile of each tumor, providing better results with lower morbidity in NSCLC patients. This strategy will become more precise with the development of real-time analysis, enabling the assessment of genetic mutations at surgery. In the near future, it is believed that patients with NSCLC may have staging and treatment based on a system called TNMB (tumor, nodes, metastasis, biology), wherein molecular aspects will be considered[36].

Risk factors and smoking

Different situations are classified as risk factors for developing lung cancer, among them family history and air pollution found in large urban centers. In some work environments such as mines, mills, textile factories, shipyards, among others, workers are exposed to carcinogenic agents, which can be cited as exposure to asbestos, radon gas, arsenic, beryllium, cadmium, silica, vinyl chloride, nickel and chromium compounds, coal products, mustard gas and radioactive minerals, such as uranium and diesel engine exhaust. Individuals that have diets lacking in fruits and vegetables, as well as those who have chronic obstructive pulmonary disease are at increased risk of developing the disease[1,39]. Unquestionably, the most important cause for the high incidence of LC in the world population is smoking.

The devastating effects of smoking on health is well established, but despite worldwide efforts aimed at reducing the prevalence of smoking, more than 1.1 billion people continue to smoke, representing one-sixth of the world population consuming around six trillion cigarettes annually[40]. Approximately half of all smokers will present some serious illness associated with smoking, such as chronic obstructive pulmonary disease or cardiovascular disease. Moreover, between 1 and 5% of smokers develop some type of malignancy, principally lung adenocarcinomas or other epithelial cell tumors[41].

In addition to LC, smoking can also cause cancer of the esophagus, oropharynx, larynx, hypopharynx and also the oral mucosa, as well as cancers of the pancreas, renal pelvis and bladder. Additionally, cigarettes have also been associated with cancer of the nose, stomach, colon, kidney, uterus, cervix, liver and myeloid leukemia[42-43]. Cigarettes contain more than 4.500 chemical compounds, including carcinogens (as polycyclic aromatic hydrocarbons, N-nitrosamines, aldehydes, benzene and aromatic amines), toxins (such as carbon monoxide, ammonia, acetone, nicotine and hydroquinone) and oxidants (superoxides and nitrogen oxides)[41]. These substances are inhaled directly or as combustion products at high temperatures.

Nicotine is responsible for maintaining smoking habits, but is not considered a carcinogen, although it

may induce tumorigenesis under certain conditions, such as hyperoxia[44]. The cigarette is configured as a disastrous instrument for providing nicotine, which is accompanied by more than 60 carcinogens[45]. Although these agents are at very low concentrations, the cumulative dose over years of smoking becomes substantial. The final effect of the influence of smoking on the body and, in particular, in the structural elements of the respiratory tract depends on, among others, local homeostasis, genetic predisposition, the local character of the cytokine network and possible pathological conditions[46].

Some of the intermediates formed by the interaction of carcinogens from cigarettes with cytochrome P450 enzymes are reactive, usually presenting an electrophilic center. These intermediates or metabolites may react with DNA, leading to the formation of covalent-bonded products, called DNA adducts, central agents in carcinogenesis[3,47]. Individuals differ with respect to DNA repair, and added to this, such a repair system is not completely effective, thus the presence of persistent DNA adducts may cause miscoding[3].

Cigarette smoking causes an increase of the excretory function of the bronchial epithelium, and have been noted in high concentrations of peptides, amines, MHC expression, and proinflammatory cytokines such as interleukin (IL)-8, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF). There is also an increase in inflammatory cells in the bronchial walls as a consequence of smoking, which results in elevated expression of adhesion molecules, increased permeability and loss of cell integrity[48,50]. Exacerbation of inflammatory process of the bronchial walls facilitates invasion of microorganisms, since disruption of cell integrity alters the profile of the cytokine network. Typical histological changes occur in the airways of smokers, resulting from the variation in the degree of loss of ciliated epithelium: an increase in the number of goblet cells, submucosal gland hypertrophy and squamous cell metaplasia[49,51]. Chronic exposure to cigarette smoke also promotes a reduction in the ciliary beat frequency and interferes with adequate clearance of the airways[46].

The number of alveolar macrophages (AM) is increased in bronchoalveolar lavage of smokers and exposure to cigarette smoke leads to changes in the phenotype of macrophages[52] as well as significant reductions in their phagocytic and antigen-presenting functions[53-54]. *In vitro* studies comparing AM of non-smokers and healthy smokers demonstrated that macrophages from smokers showed lower bacteriostatic or bactericidal properties than those of non-smokers[55]. In addition, smoking promotes the activation of the production of proinflammatory mediators, reactive oxygen species and proteolytic proteins and enzymes, thus promoting a cell mechanism linking smoking to inflammation and tissue damage[56-57]. Generally MA obtained from smokers are less mature, show elevated CD14 expression (monocyte marker), condensed cytoplasm, are hyperdense and have diminished IL-1, IL-6, IL-8 and TNF-α secretions[58-59].

Exposure to cigarette smoke leads not only to increased influx of macrophages, but also neutrophils in the airways. The function of these cells becomes disordered in smokers when compared with non-smoking individuals, which eventually leads to compromise of the body's defenses[60]. The increase in the number of neutrophils in the airways culminates with elevated levels of proteolytic enzymes, such as neutrophil elastase, cathepsin G, and proteinase 3. These proteases stimulate the release of mucin from goblet cells, aside from their destructive effect on ciliated cells and the extracellular matrix[46]. A study evaluating the *in vitro* treatment of neutrophils with cigarette smoke extract observed a dramatic suppression of caspase-3-like activity that culminated in reduced phagocytic activity[61]. Furthermore, in another study in which neutrophils from healthy non-smokers were incubated with nicotine, the authors also observed the capacity to compromise phagocytosis exerted by these cells, in part due to the decrease in the ability in forming actin filaments due to interference in calcium signaling[62].

Smoking causes diminished cytotoxicity and cytokine production by natural killer (NK) cells in humans and rats[63]. Studies have also shown the presence of eosinophils in the submucosa of bronchial biopsies from smokers[64]. Moreover, research with rats have shown that $CD8^+$ T cells undergo oligoclonal expansion in animals that have been chronically exposed to cigarette smoke, and persists six months after the interruption to smoke exposure[65]. Other studies have correlated the infiltration of $CD8^+$ T cells in bronchial biopsy and determined symptoms reported by healthy smokers described as "problems with breathing"[66].

CONCLUSION

Against the alarming backdrop of incidence and mortality of LC, it is necessary to deepen the understanding of the cell and molecular mechanisms that involve the development of this malignant disease. Understanding the pathogenesis of smoking, the main risk factor for the onset of LC, is still a subject of great interest, since one-sixth of the world´s population consumes about six trillion cigarettes annually.

Lung tumors have nuances that make it quite intriguing and aggressive, as histologic heterogeneity and difficulty in early detection. Additionally, unlike colon and breast tumors, which have seen increasing cure rates, LC remains with the fixed rate of 15%, a fact that contributes to the greater lethality of this carcinoma.

All these factors point to the need to continue and deepen the studies that aim at early detection and the development of new specific therapeutic strategies, allowing for a more individualized treatment in patients with LC. Therefore, we believe that such events would

result in higher cure rates and better quality of life for patients with this malignancy, which is the leading cause of cancer death worldwide.

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