Focus on lung cancer

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Epidemiology and incidence statistics

Lung cancer is the most common form of cancer in the world (12.3% of all cancers), with an estimated 1.2 million new cases in 2000 (Parkin et al., 2001). Tobacco smoking is the most important cause of lung cancers with 80%–90% arising in cigarette smokers (Figure 1). There are major geographic, racial, and gender differences in incidence and some reports suggest that women may be at increased risk of lung cancer from exposure to tobacco smoke carcinogens. A lifetime smoker has a 20- to 30-fold increased risk of developing lung cancer compared to a lifetime nonsmoker. While smoking prevalence is decreasing in the USA, in China and Eastern Europe, there is an epidemic of smoking which will result in tens of millions of new cases in this century (Parkin et al., 2001; Peto et al., 1999, 2000). Thus, lung cancer is the most preventable of all cancers, and smoking cessation results in decreased risk after a lag period of ~7 years (Peto et al., 1999). However, this decreased risk never reaches baseline levels, and lung cancer in the USA is becoming a disease of former smokers. Despite improvements in therapy, ~90% of lung cancer patients will die from their disease. In 2000, it is estimated that lung cancer resulted in 1.1 million deaths worldwide, or 17.8% of all cancer deaths. However, only ~11% of heavy cigarette smokers ultimately develop lung cancer, suggesting that there may be genetic factors predisposing to lung cancer risk (Lippman and Spitz, 2001).

Genetic epidemiology

Segregation studies indicate a 2.5-fold risk of lung cancer from family history after controlling for cigarette smoking, suggesting the presence of a rare autosomal dominant gene predisposing to lung cancer (Amos et al, 1999). The familial association is most readily detected in lung cancer occurring in nonsmokers. However, the majority of inherited lung cancer risk probably comes from small but significant effects arising from genetic polymorphisms that are frequent in the population (Amos et al, 1999). Tobacco smoke contains over 20 known lung cancer-specific carcinogens, especially the polycyclic aromatic hydrocarbons and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Polymorphisms that reduce the activity of the glutathione-S transferase family, which inactivates the carcinogens, or that increase the activity of the P450 family, which activates them, may result in increased cancer susceptibility. The activated carcinogens bind to DNA, forming adducts, leading to mutations, especially G-to-T transversions, which may be repaired, lead to apoptosis, or persist. Molecular epidemiology has shown differences in smoking-related risk based on the interactions between tobacco carcinogens, genetic polymorphisms involved in activating and detoxifying these carcinogens, and host cell efficiency in monitoring and repairing tobacco carcinogen-DNA damage (Fong et al., 2001).

Conventional diagnostics and therapeutics

The majority of lung cancer cases are diagnosed when a symptom appears related to the primary, metastatic disease, or paraneoplastic syndrome (Minna, 2000). The evaluation of the lung cancer patient involves obtaining material for a histologic diagnosis, determining the stage of the disease based on the International TNM staging system using standard physical examination, computed tomography (CT) scans of the chest and abdomen, pulmonary function tests, routine laboratory tests, and weight loss of the patient. Often a flexible fiberoptic bronchoscopy is performed to directly look at the large airways and obtain biopsy material, and a CT guided fine needle biopsy is often performed.

Four major histologic types comprise the majority of lung cancers, and include small cell lung cancer (SCLC) and the three non-small cell lung cancer (NSCLC) types. Squamous and SCLC arise mainly from the central airways, while adenocarcinomas (including bronchioloalveolar cancer) are peripherally located. Large cell lung cancer represents less differentiat-

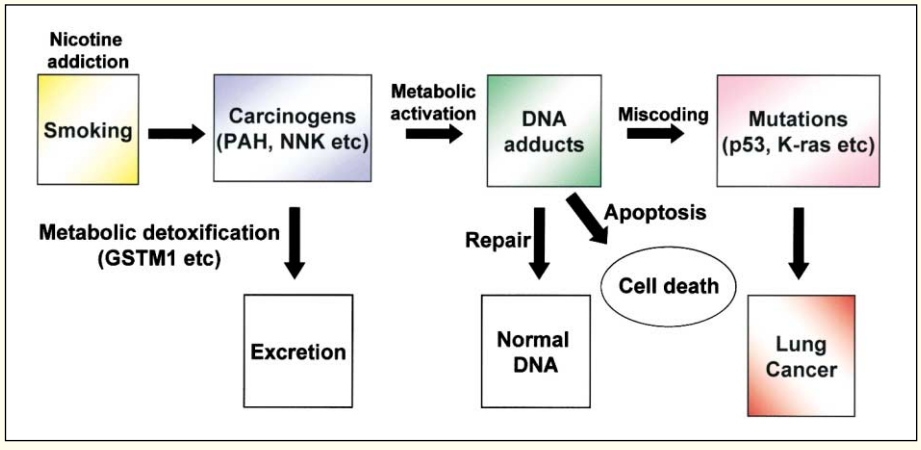

Figure 1. Nicotine addiction, tobacco smoke carcinogens, and the pathogenesis of lung carcinomas

Nicotine addiction is the powerful engine that prevents smokers from quitting. The many lung cancer-specific carcinogens (including polycyclic aromatic hydrocarbons and nitrosamines) in the particulate matter of tobacco smoke have to be metabolized before they are either secreted or can bind to DNA with the formation of adducts. DNA adducts may be repaired or lead to apoptosis. If they persist, miscoding mutations in key genes such as P53 or RAS may cause genetic instability, leading to further mutational damage and eventually to cancer. Modified from Hecht (1999).

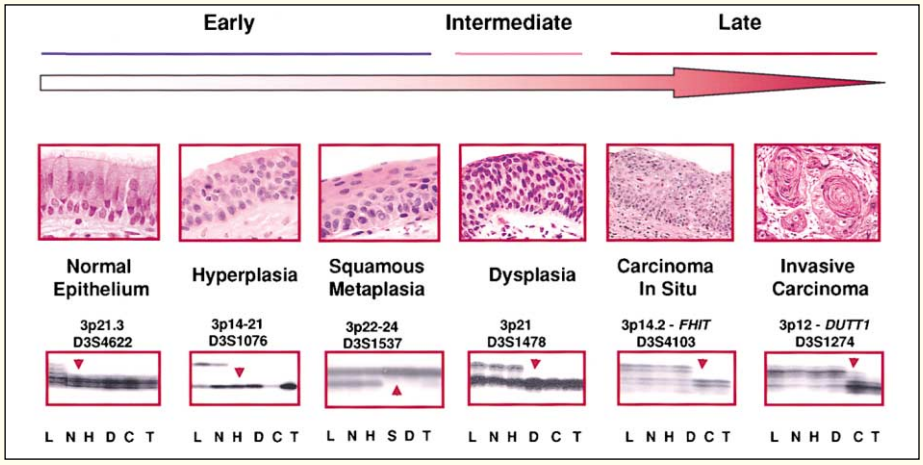

Figure 2. Progressive morphological and molecular changes during the multistage development of squamous cell lung carcinomas

Progressive allelic losses at the short arm of chromosome 3 (3p) are illustrated. Each panel represents a resected squamous cell carcinoma. All of the preinvasive lesions were identified and carefully microdissected and analyzed for loss at different 3p loci using multiple polymorphic markers. Losses at some sites (such as 3p21) occur early, while losses at other sites (such as 3p12) occur late (Wistuba et al., 1999). The lesion at which the earliest allelic loss occurred for each of the markers in each case is identified. L = lymphocytes (a source of constitutional DNA); N = histologically normal epithelium; H = hyperplasia; D = dysplasia; C = carcinoma in situ; T = invasive tumor. We thank Ivan Wistuba for assistance in preparation of the figure.

ed forms of the other NSCLC types. There is no squamous epithelium in the normal lung, and these tumors arise from metaplastic changes resulting from smoking. SCLCs have neuroendocrine features and arise from cells programmed to differentiate along these lines. Adenocarcinomas arise from the progenitor cells of the bronchioles (Clara cells) or alveoli (Type II pneumocytes) or from mucin producing cells. Adenocarcinoma is the most common form of lung cancer in the world today, and its frequency is increasing rapidly. It is by far the commonest form in women, never smokers and in young people.

Patients with SCLC with disease confined to the chest ("limited stage") receive concurrent chest radiotherapy and chemotherapy that often includes cisplatin and etoposide while those with more "extensive stage" disease receive chemotherapy alone with drug combinations such as cisplatin, etoposide, irinotecan, topotecan, or paclitaxel. These treatments lead to long-term survival and potential cure in 10%–25% of limited stage patients and a few extensive stage patients, while nearly all of patients have their tumors shrink with therapy, gain symptom relieve, and an increase in median survival of 10–16 months (Minna, 2000).

NSCLC patients with disease confined to the chest are further staged by surgical evaluation of the mediastinum for lymph node involvement (and more recently by positron emission tomography "PET" scans). Those patients without evidence of mediastinal lymph node involvement or distant metastatic disease have a surgical resection of the primary tumor (involving a lobe of the lung or the entire lung). For those patients with mediastinal lymph node involvement either preoperative chemotherapy ("neoadjuvant chemotherapy") followed by an attempt at surgical resection or a combination of chemotherapy and chest radiotherapy is given in a curative approach. Alternatively, high dose radiotherapy to the chest is given with curative intent. Depending on the stage of the disease, surgical based approaches result in long-term disease free survival and potential cure of ~30%–50% of resected patients. Unfortunately, the majority of patients end up having distant metastatic disease. Currently available chemotherapy agents for the treatment of NSCLC include doublet combinations of cisplatin or carboplatin, and etoposide, gemcitabine, paclitaxel, docetaxel, vinorelbine, and irinotecan (Bunn, 2001). These combinations produce 1-year survival rates of about 35% and 2-year survival rates of about 15% (Bunn, 2001). While these therapies are not curative they often result in symptom relief and prolongation of life in the order of 2–10 months. Any patient not controlled by chemotherapy is a candidate for local radiation therapy to relieve symptoms.

Key genes and pathways involved in the pathogenesis of lung cancer

Oncogenes that contribute to the pathogenesis of lung cancer include c-*myc*, mutated K-*ras* (15%–20% of NSCLC, predominantly adenocarcinomas but never in SCLC) and overexpressed EGFR, cyclin D1, and BCL2 (Fong et al., 2001). Telomerase RNA (hTR) and the catalytic component (hTERT) are expressed in nearly all lung cancers providing a mechanism for cellular immortality. Tumor suppressor genes (TSGs) frequently involved include p53 (~90% of SCLC; 50% NSCLC), Rb (~90% SCLC; ~20% NSCLC), and p16 (>50% NSCLC; <1% SCLC). Abnormalities in the function of FHIT, RASFF1A, and SEMA3B are implicated in the pathogenesis of a number of lung cancers (Zochbauer-Muller et al., 2002), irrespective of their controversial label as TSGs. Other TSGs that are less frequently involved but whose occasional inactivation points to involvement of their associated cell regulatory pathways include *PTEN*, *hOGG1* (DNA repair), *BAP1* (ubiquination). The major mode of inactivation of expression of many genes (*APC*, *CDH13*, *RARβ, FHIT*, *RASSF1A*, *TIMP3*, *p16*, *MGMT*, *SEMA3B*, *DAPK*) involves tumor-acquired promoter hypermethylation (Belinsky et al., 1998; Zochbauer-Muller et al., 2001). The methylated sequences can be detected in tumors, smoking damaged normal lung (preneoplastic changes), sputum, and blood (Zochbauer-Muller et al., 2001). These represent attractive surrogate biomarkers for early detection and monitoring chemoprevention, smoking cessation and response to therapy. Mitochondrial DNA, especially the displacement (D) loop, also undergoes mutations, in lung cancer, but the functional significance of this is unknown. The cell regulatory pathways that are deregulated in lung cancer include the Rb/p16/cyclin D1, p53/MDM2/p19^{ARF}, wnt/APC, EGFR/Ras, PP2a, and telomerase pathways, as well as several genes involved in various DNA repair pathways. In addition, there is evidence of deregulation of apoptosis, angiogenesis, autocrine/paracrine growth factor circuits. The methylation changes (epigenetic alterations) and genome wide allelotyping changes showing allele loss (genetic alterations) are starting to provide speculative estimates for the minimal number of events (20–30) leading to the development

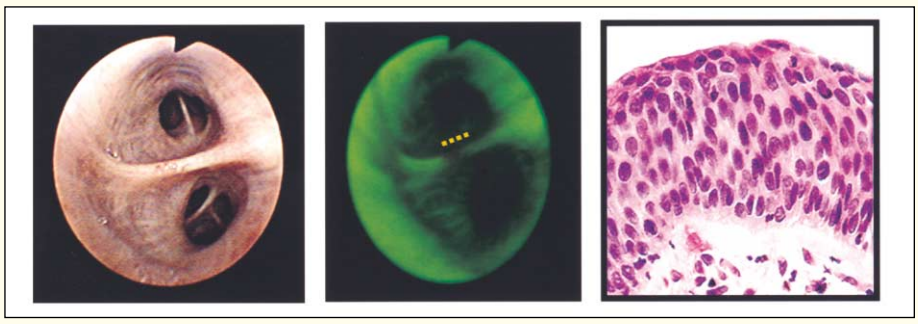

Figure 3. Fluorescence bronchoscopy for the identification of preinvasive and early invasive lesions

Decreased autofluorescence may identify preinvasive or early invasive lesions that conventional white light bronchoscopy (left) failed to identify. A small focus (less than 2 mm in size) of decreased fluorescence (middle) is identified in the spur of a major bronchus (as marked by the dotted line; Lam et al., 1998). Biopsy of this lesion (right) demonstrated moderate to severe dysplasia. We thank Stephen Lam for providing the images.

of clinically evident lung cancer (Zochbauer-Muller, 2001; Girard, 2000). Many of the changes are common between NSCLC and SCLC while others have a predominance for one or the other.

Molecular abnormalities in smoking-damaged lung as preneoplastic lesions

Lung cancers arise after a series of molecular and morphological events. The molecular events commence in histologically normal epithelium and show a specific sequence (Hirsch et al., 2001). Precise microscopic based microdissection of epithelial tissue followed by allelotyping of smoking damaged lung from lung cancer patients or current or former smokers without lung cancer revealed hundreds of thousands of lesions (~90,000 cells in size) containing clonal abnormalities of allele loss ("a field defect"), occurring in both histologically normal as well as abnormal (e.g., hyperplasia, dysplasia, or carcinoma in situ; Figures 2) respiratory epithelium (Park et al., 1999). While these changes are found in the lungs of current and former smokers without lung cancer they are almost never found in life time never smokers (Wistuba et al., 1997). These clonal changes persist for decades after smoking cessation (Wistuba et al., 1997). There is a preferred order of these allele loss changes with 3p allele loss (several 3p sites) followed by 9p (*p16* locus) as the earliest changes occurring in histologically normal epithelium (Wistuba et al., 1997, 1999, 2000). Similar evidence exists for multiple promoter methylation changes in smoking damaged lung and sputum (Zochbauer-Muller et al., 2001). Considerable attention has been given to the identification of the 3p genes involved in lung cancer pathogenesis including *RARβ* at 3p24, *FHIT* at 3p14.2, *RASSF1A*, *FUS1*, and *SEMA3B* located together at 3p21.3, and potentially *ROBO1* at 3p12 (Wistuba et al., 2000; Damman et al., 2000; Burbee et al., 2001; Tomizawa et al., 2001; Zochbauer-Muller et al., 2002). Their expression is frequently lost in lung cancer, usually by promoter methylation.

Recent advances in diagnosis, treatment, and prevention

Spiral computed tomography scans for early detection of lung cancer

CT (computed tomography) scans have been routinely used in the staging of lung cancer patients. Recently there has been intense interest generated in using low dose spiral (also called helical) computed tomography scans to screen asymptomatic patients with a history of cigarette smoking for the early detection of lung cancer (Henschke et al., 1999). The spiral CT screening trials conducted primarily in the USA and Japan have not yet been evaluated in randomized clinical trials but detected ~30 lung cancers per 1000 patients undergoing an initial screen. Nearly 85% of these tumors were very early stage NSCLCs (usually adenocarcinomas) (stage I) and presumably will have a very high rate of cure by surgery alone. Debate has ensued over whether these cancers represent "over-diagnosis"; that is tumors that would never grow to kill the patient, and thus whether prospective randomized trials should be conducted. Autofluorescence bronchoscopy, when used as an adjunct to standard white light bronchoscopy, enhances the bronchoscopist's ability to localize small preneoplastic and neoplastic lesions (Figure 3) (Lam et al., 1998).

PET (positron emission tomography) scans for improved staging of lung cancer

A major advance in lung cancer staging has been the introduction of ^{18}F-2-fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) (Pieterman et al., 2000). FDG PET imaging uses the increased accumulation of FDG in tumor cells compared to normal cells to identify malignant lesions and is ~95% sensitive and ~85% specific. PET is useful for detection of both mediastinal and distant metastatic disease and whole body PET scans frequently detect unsuspected distant metastatic disease or show other lesions to be benign resulting in clinical management changes in up to 40% of patients. PET is also used in evaluating solitary pulmonary nodules and can document responses to chemotherapy in a matter of days.

Molecularly targeted therapies

New systemic therapies directed against rational targets have entered clinical trials for the treatment of lung and other cancers (Gibbs, 2000). They include angiogenesis inhibitors, epidermal growth factor receptor (EGFR) inhibitors, HER2/neu receptor inhibitors and other tyrosine kinase inhibitors, inhibitors of Ras activation and function (such as farnesyltransferase inhibitors), matrix metalloproteinase inhibitors, cyclin dependent kinase inhibitors, inhibitors of autocrine and paracrine growth factor loops, anti-sense molecules such as those directed against BCL2 or PKCα, vaccines against tumor mutated or specifically expressed peptides, and gene replacement therapy. While currently available chemotherapy has beneficial effects, often this is small. Recently, this rationally targeted approach has been greatly stimulated by the clinical success of BCR/Abl and c-kit inhibitors in chronic myelogenous leukemia and gastrointestinal stromal tumors.

Prevention of lung cancer

The primary prevention of lung cancer through the prevention of smoking initiation (particularly in children and young adults) and helping people to stop smoking remains the long term key to lung cancer control. There have been significant decreases in

smoking in certain segments of the population (such as in adult white males) that have had effects in leveling off the increase in lung cancer incidence. Whether there are gender differences in response to tobacco carcinogens, in development of nicotine addiction, or in expression of autocrine growth factor receptors such as for gastrin releasing peptide is an important new area of research. Trial proven aids for smoking cessation include the involvement of physicians in patient counseling, psychological support, nicotine replacement therapy, and the use of antidepressants (Fiore et al., 1997). Persons may also have inherited differences in their susceptibility to nicotine and thus smoking addiction.

Treatment of current and former smokers with agents thought to block the progression of lung cancer pathogenesis (chemoprevention) has been fraught with difficulty. There have been definitive, randomized, controlled lung-cancer chemoprevention trials all of which produced negative (either neutral or harmful) primary end point results (Lippman and Spitz, 2001). Recently vitamin E and selenium were found to show promise for lung cancer prevention, and there is interest in testing Cox-2 inhibition or the use of EGFR and farnysltransferase inhibitors.

Future challenges

The major challenges are: to develop social, cultural, and economic approaches to deal with the tobacco epidemic; develop new methods to prevent smoking initiation and to aid in smoking cessation including understanding the genetic basis of nicotine addiction; to develop new imaging, molecular genetic, and genetic epidemiological methods for early detection and the chemoprevention in lung cancer particularly in those individuals who have altered their life styles by stopping cigarette smoking; to be able to delineate all the significant molecular abnormalities present in any one patient's tumor and to use this information for early molecular detection, prediction of biological/clinical behavior and prognosis, and selection or rational development of therapeutics.

Acknowledgments

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