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REVIEW ARTICLE

Lung Cancer in Never Smokers: A Review

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ABSTRACT

Lung cancer is the leading cause of cancer-related death in the United States. Although tobacco smoking accounts for the majority of lung cancer, approximately 10% of patients with lung cancer in the United States are lifelong never smokers. Lung cancer in the never smokers (LCINS) affects women disproportionately more often than men. Only limited data are available on the etiopathogenesis, molecular abnormalities, and prognosis of LCINS. Several etiologic factors have been proposed for the development of LCINS, including exposure to radon, cooking fumes, asbestos, heavy metals, and environmental tobacco smoke, human papillomavirus infection, and inherited genetic susceptibility. However, the relative significance of these individual factors among different ethnic populations in the development of LCINS has not been well-characterized. Adenocarcinoma is the predominant histologic subtype reported with LCINS. Striking differences in response rates and outcomes are seen when patients with advanced non–small-cell lung cancer (NSCLC) who are lifelong never smokers are treated with epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitors such as gefitinib or erlotinib compared with the outcomes with these agents in patients with tobacco-associated lung cancer. Interestingly, the activating mutations in the EGFR-TK inhibitors have been reported significantly more frequently in LCINS than in patients with tobacco-related NSCLC. This review will summarize available data on the epidemiology, risk factors, molecular genetics, management options, and outcomes of LCINS.

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INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths in the United States.[1] A review of cancer distribution on a global scale in 2002 found lung cancer to be the most commonly diagnosed cancer annually since 1985.[2] The global distribution of lung cancer has undergone major changes, with reduction in the number of cases in the developed world. However, the proportion of lung cancer patients in developing nations has increased from 31% to 49.9% in the last two decades.[3] It has recently been estimated that 15% of men and 53% of all women with lung cancer worldwide are never smokers.[3] There is a renewed interest in the problem of lung cancer in never smokers (LCINS) after the observation that the response rates with epidermal growth factor receptor (EGFR) tyrosine kinase (TK) inhibitors, such as gefitinib and erlotinib, are higher in never smokers than in smokers with advanced non–small-cell lung cancer (NSCLC). Moreover, activating mutations in the EGFR-TK domain have been reported more frequently in never smokers than smokers with NSCLC.[4] This review will summarize the current body of knowledge on LCINS.

SEARCH STRATEGY AND SELECTION CRITERIA

The search strategy selected articles from MEDLINE using the PubMed system. Articles published within the last 10 years were considered for review whenever possible. The key words used to search were "never smokers," "non smokers," "lung cancer," "NSCLC" cross referenced with "epidemiology," "risk factors," "molecular biology," "EGFR," "erlotinib," "gefitinib," and "survival." Only articles with abstracts in English were considered. In addition, relevant articles that were quoted in publications from the original search results were also reviewed.

EPIDEMIOLOGY

Tobacco smoking accounts for more than 90% of lung cancers in men and 75% to 85% of lung cancers in women in the United States and European Union.[3,5,6] Although these figures are similar in Asian men, the proportion of Asian women with lung cancer who smoke tobacco is much lower. These findings were predominantly from Asian populations in the Pacific Rim countries, with minimal or no information from other parts of Asia. The proportion of women with lung cancer who reported tobacco smoking varies from region to region even within Asia, from 25% in Korea to 56% in Hong Kong.[5,7-9]

A review of 16 studies before 1990 substantiates the common observation that adenocarcinoma is the most common histologic type in LCINS.[5] This finding has been confirmed subsequently (Table

1).[10-15] The predominant distribution of adenocarcinoma histology is seen globally. Because small-cell lung cancer is almost exclusively related to tobacco smoking, this review will focus on NSCLC in never smokers.

ETIOLOGIC FACTORS IN THE DEVELOPMENT OF LCINS

Environmental Tobacco Smoke

Given the dominant role of tobacco smoking in the development of lung cancer, environmental tobacco smoke (ETS) has naturally aroused considerable interest in the etiopathogenesis of LCINS.[13,23-29] ETS is defined as "sidestream smoke from the smoldering tobacco between puffs and exhaled mainstream smoke from the smoker."[25] The association between ETS and lung cancer in spouses of tobacco smokers was first reported 25 years ago.[30] This was followed by several epidemiologic studies on the association of ETS and lung cancer. In 1992, the Environmental Protection Agency published a review of the epidemiologic studies on the effect of ETS.[31] It reported that ETS is associated with increased risk for lung cancer and that it accounts for 3,000 lung cancer deaths per year in the United States. However, the report did not provide a more accurate quantification of the risk of LCINS from ETS and it included former smokers in the nonsmoker category. In addition, many of the studies reviewed were limited by small sample sizes and by misclassification and selection bias. The National Cancer Institute's 10th *Smoking and Tobacco Control Monograph* reviewed studies published between 1991 and 1997 in the United States, Europe, and Asia.[32] It included studies on ETS exposure from spouses and the workplace and exposure in other social settings. They concluded that ETS exposure resulted in an excess risk of 20% for developing LCINS. Compared with the 1992 Environmental Protection Agency report, this study included studies with larger sample sizes and studies that controlled for the potential effects of bias.

The International Agency for Research on Cancer estimated the increased risk for developing lung cancer from ETS exposure to be 35% in men and 25% in women when compared with men and women not exposed to ETS.[23,33] In a separate meta-analysis of 19 studies exclusively on never-smoking women, the increased risk as a result of ETS was estimated to be 20%.[23]

In 2005, Vineis et al[25] published their nested case-control study on the European Prospective Investigation into Cancer (EPIC) and nutrition cohort population. The EPIC population consisted of more than 500,000 volunteers from 10 European countries enrolled between 1993 and 1998. This study included 123,479 never or former smokers (95,947 were women) in the EPIC cohort with data on exposure to ETS, of whom 97 developed lung cancer. The hazard ratio for lung cancer for the whole cohort exposed to ETS was 1.34 (95% CI, 0.85 to 2.13), and for never smokers (102,923 of the 123,479 never or former smokers), the hazard ratio was 1.05 (95% CI, 0.60 to 1.82). This study did not detect a statistically significant hazard ratio for developing lung cancer from ETS.

It is likely that the modest risks posed by ETS require larger sample sizes to detect a statistically significant relationship. Thus, current evidence suggests that ETS plays, at best, only a modest role in the development of LCINS.

Exposure to Cooking Fumes

The low incidence of tobacco smoking in Chinese women who develop lung cancer led to the search for other potential risk factors. Factors that may play a role in the etiology of LCINS include the type of cooking (deep frying *v* stir frying), presence or absence of fume extractors, and duration of total cooking years.[17,19,34,35] A case-control study of 672 women with lung cancer (65% never smokers) and 735 controls identified rapeseed oil fumes to be associated with increased risk for lung cancer.[36] Stir frying more than 30 dishes per week was associated with high risk (relative risk, 2.6; 95% CI, 1.3 to 5.0). However, exposure to coal or other fuel fumes was not associated with higher risk. Cell line experiments have reported emissions from heated rapeseed and soybean oil to be mutagenic.[37] Several case-control studies continue to identify cooking oil fumes as a risk factor for lung cancer in Chinese women.[17,19,38,39]

Exposure to coal fumes is reported to be associated with increased risk for lung cancer in Chinese women.[35,40-42] A case-control study (965 patients and 959 controls) on Chinese women reported an increased risk for lung cancer (relative risk, 1.5; 95% CI, 1.1 to 2.0)

Table 1. Distribution of Histologic Types of LCINS

Reference	Region	No. of Patients	Histologic Type (% of patients)				
			Adenocarcinoma	Bronchioloalveolar	Squamous	Large Cell	Small Cell
Fontham et al[13]	United States	653	76	—	10*	11	—
Toh et al[16]	Singapore	286	70	—	6	—	—
Yu et al[17]	Hong Kong	200	68	—	4	4	—
Brownson et al[14]	United States	328	67	5	3	—	0.9
Kreuzer et al[18]	Germany	118	64	—	12	—	11
Ko et al[19]	Taiwan	106	65	—	17	3	15
Kabat and Wynder[11]†	United States	134	62	12	16	4	5
Stockwell et al[20]	United States	210	61	—	17	—	7
Boffetta et al[21]	Europe	650	51	—	27*	—	—
Kubik et al[22]	Czech Republic	51	48	2.1	21	2	4
Dibble et al[15]	United States	180	47	—	11	—	—
Gürsel et al[8]	Turkey	114	40	—	13	3	21

Abbreviation: LCINS, lung cancer in never smokers.
*Includes small-cell carcinoma.
†Kreyberg classification.

with the use of heating Kang, a form of indoor heating that uses coal.[35] However, the same study did not detect a significant risk in persons exposed to fumes from coal stoves or coal burners. A more recent study detected increased risk for lung cancer in both men and women when exposed to burning coal as indoor heating fuel over a period of 30 years.[42] The odds ratio (OR, 1.29; 95% CI, 1.03 to 1.61) was significant after adjusting for smoking and socioeconomic status ($P = .02$). However, there have been other studies reporting no increased risk for lung cancer from coal fumes.[19,36,39] A few other studies that reported higher risk either did not achieve significance or had small sample size.[43-47]

However, these case-control studies are inherently affected by recall bias, inaccurate methods for measuring exposure, and possibly confounding from other carcinogens such as fumes from cooking fuels, ETS, and radon. Cooking fumes, particularly from frying, contain proven carcinogens, and the resulting indoor pollution from these fumes may increase the risk for lung cancer. Simple public health measures, such as proper venting of smoke and reduction or avoidance of certain cooking methods to reduce indoor pollution, could prevent lung cancer in select populations where old-fashioned methods of cooking are still in use and the ventilation is suboptimal. However, the evidence for indoor coal fumes is more controversial and requires additional evidence to be considered as a major risk factor for lung cancer.

Inherited Genetic Susceptibility

The role of inherited genetic factors in the development of tobacco-related carcinogenesis is being actively studied. However, only a few studies have examined the role of inherited susceptibility in LCINS.

The role of inherited susceptibility in women with LCINS was examined in a case-control study.[48] The presence of a family history of respiratory tract cancer in first-degree relatives (> two thirds of relatives had lung cancer) conferred an excess risk of 30% (n = 646; 95% CI for OR, 0.9 to 1.8). The familial association was stronger in the subset of patients with adenocarcinoma of the lung (OR, 1.5; 95% CI, 1.0 to 2.2).[48] In another retrospective study of 257 LCINS patients and 277 never-smoking controls, the risk for developing lung cancer among never smokers who had a positive family history of lung cancer was significantly greater among those in the age group of 40 to 59 years (OR, 7.2; 95% CI, 1.3 to 39.7) than for the entire study cohort (OR, 1.4; 95% CI, 0.8 to 2.5).[49] The increased risk in the younger age group suggests the possibility of genetic influence that manifests early in life. However, the CIs are wide. A family history of lung cancer was associated with significant risk for LCINS in a study involving 216 Taiwanese female never smokers (OR, 5.7; 95% CI, 1.9 to 16.9).[50] The risk was higher if the relative was a woman. The association between familial history of lung cancer and LCINS seems to be stronger in patients younger than 60 years at presentation and patients with adenocarcinoma.

The available evidence supports the view that family history of lung cancer is associated with increased risk for lung cancer in both smokers and never smokers, and the autosomal codominant gene model seems to be the best fit in explaining familial clustering of lung cancer.[51,52] On the basis of an analysis of 52 families (that included tobacco smokers) with at least three members diagnosed with lung, laryngeal, or throat cancer, investigators identified a major susceptibility locus on chromosome 6q23-25.[53] This particular area in chromosome 6 includes more than 100 genes, and some of them are potential candidates for the role of a lung cancer susceptibility gene. It is conceivable that even brief exposure to tobacco smoke (such as ETS) would result in a disproportionately high risk in obligate carriers for developing lung cancer.[54] Identification of this gene would obviously represent a major breakthrough in lung cancer.

A recent report identified germline transmission of EGFR *T790M* gene mutation in members of a European family diagnosed with primary NSCLC. In addition, secondary activating EGFR-TK mutations were also detected in four of six tumors analyzed.[55] The *T790M* mutation is reported to be associated with development of resistance to EGFR-TK inhibitors.[56,57] The presence of this mutation in a case of familial clustering of NSCLC (predominantly bronchoalveolar carcinoma) indicates that *T790M* mutation may mediate changes in the EGFR signaling pathway and that these alterations could have a role in the inherited susceptibility to NSCLC. However, these findings require further confirmation.

Genetic polymorphisms affecting activating and detoxifying enzymes play an important role in lung cancer carcinogenesis. Polycyclic aromatic hydrocarbons (PAH) in tobacco smoke are metabolized in a two-phase process; phase I is when the PAH is activated by cytochrome P450s (CYPs), and phase II involves detoxification by glutathione *S*-transferases (GSTs). The activation phase results in the metabolic intermediates that can cause DNA adduct formation. Phase II involves detoxification of these carcinogenic metabolic intermediates.[58]

CYP1A1 enzyme is involved in the activation of procarcinogens in tobacco smoke.[59] In a pooled analysis of 11 studies with 1,950 patients and 2,617 controls, the polymorphism in exon 7 of the *CYP1A1* gene was found to be associated with increased risk for lung cancer.[60] Furthermore, subset analysis detected an association between LCINS and CYP1A1 polymorphism in exon 7 (n = 48; OR, 2.06; CI, 1.36 to 3.13; $P = .008$). Because this was a pooled analysis of different studies, it is likely that this study could have been affected by variation in the ETS exposure, PAH exposure from sources other than tobacco smoking (eg, air pollution), and misidentification of tobacco smokers as never smokers. *GSTM1* null genotype (null genotype is homozygous deletion of the gene resulting in no enzyme activity for the important detoxification process) was associated with a slightly increased risk for LCINS in Japanese women (n = 158); the OR was 1.37 (95% CI, 0.90 to 2.09). This study analyzed the role of ETS in this genotype. Women with this genotype and heavy ETS exposure (a spouse with > 40 pack years of tobacco smoking) had a significantly elevated risk (OR, 2.27; 95% CI, 1.13 to 4.57) compared with women living with a spouse with less than a 40–pack year history of tobacco smoking and/or who had *GSTM1* non-null (heterozygote or wild type) genotype.[61] An earlier study from the United States reported that *GSTM1* null genotype in never-smoking women increased their risk for lung cancer when exposed to ETS.[62] The evidence from these studies suggests a possible influence of genetic polymorphisms in the development of LCINS, and this is an area that certainly deserves further diligent investigation.[60-62]

Occupational and Environmental Exposure

Radon exposure. Radon is a uranium degradation product known to be associated with lung cancer in uranium mine workers.[63,64] Radon emits alpha particles, inducing DNA damage in respiratory epithelial cells. Alpha particle radiation is associated with

inactivation of the *p16* tumor suppressor gene by inducing methylation.[65] Radon is present in the soil and air as a pollutant. Exposure to radon among the general population is believed to be associated with an increased risk of lung cancer.[66]

The Iowa Radon Lung Cancer Study investigated the role of residential radon exposure in developing lung cancer.[67] The study participants were women who had lived in the same house in Iowa for 20 years. It was a population-based case-control study with 413 lung cancer patients and 614 age-matched controls. Study participants' exposure to radon over 1 year was estimated using radon detectors at the homes of the participants, measurement of regional outdoor radon levels, and estimation of participants' radon exposure in other buildings. The authors reported excess odds of developing lung cancer of 0.50 (95% CI, 0.004 to 1.81) and 0.83 (95% CI, 0.11 to 3.34) for all participants and for live participants, respectively. They also detected a positive trend for risk of lung cancer with increasing radon exposure levels, with ORs of 1.00, 1.34, 1.73, 1.62, and 1.79 for cumulative exposures of radon measured in working level months of 0 to 4.23, 4.24 to 8.47, 8.48 to 12.70, 12.71 to 16.94, and more than 16.95. The positive trend in OR for lung cancer with increasing radon concentration was significant for categoric analysis ($P = .05$) but not for continuous analysis ($P = .14$). When the analysis was restricted to live patients and controls, the OR trend achieved significance in both categoric and continuous analysis. In addition, large-cell carcinoma was also reported to have a significant trend of increasing risk with increasing levels of residential radon exposure. This study associates residential radon exposure with increased lung cancer risk. A majority of the case-control studies conducted in the United States on residential radon exposure support this finding as well.[68-72] Thus, current evidence from the case-control studies and data from uranium miners suggests that prolonged radon exposure is associated with increased risk for lung cancer.

Asbestos. Occupational asbestos exposure is a carcinogen associated with development of lung cancer.[73] The risk for lung cancer from asbestos exposure is dependent on both fiber type and dose.[74-76] A cohort study on occupational asbestos exposure in Holland reported an increased relative risk for lung cancer of 3.5 after controlling for age, smoking, and vitamin intake.[77]

A retrospective population study from asbestos mining areas in Quebec reported that lung cancer mortality in the nonoccupational setting (women) is insignificant.[78] The mortality data for all women at least 30 years of age between 1970 and 1989 were collected. The average cumulative asbestos exposure during that period in those areas was also calculated. On the basis of the collected data, the authors did not detect a significant increase in mortality from lung cancer; the standardized proportionate mortality ratio was 1.1 (95% CI, 0.88 to 1.38). A similar Italian study on lung cancer mortality in the nonoccupational setting did not detect increased lung cancer mortality from nonoccupational asbestos exposure.[79] The results from these studies indicate that nonoccupational exposure to asbestos may not have a significant role in increasing mortality from LCINS. However, the study population was predominantly exposed to chrysotile fibers, which are larger and more rapidly cleared from the lungs than amphibole fibers. This may explain the lack of association between lung cancer mortality and asbestos exposure.[80]

Other environmental agents. Exposure to heavy metal and other carcinogenic chemical agents, such as arsenic, cadmium, nickel, metal dust, PAH, and vinyl chloride, has been thought to play a role in the etiopathogenesis of lung cancer.[81] The exact mechanism by which heavy metals cause lung cancer is not well-understood. Cadmium is believed to bind weakly to DNA and act through an epigenetic mechanism.[82,83] Nickel and chromium are known to cause oxidative stress and can generate reactive oxygen species.[84] Ionizing radiation induces mutagenic changes by direct DNA damage. PAH carcinogenic effects are through DNA adduct formation and failure of DNA repair.[85]

Hormonal Factors

Because LCINS affects women disproportionately more than men, not surprisingly there is considerable interest in exploring the role of estrogen in this disease. Estrogen receptors (ER) ERα and ERβ have been detected in both normal and cancerous lung tissues in men and women.[86-88] Furthermore, activation of the ERs increases expression of certain genes, leading to increased cellular proliferation and tumor growth.[87-89] The expression of ERα by reverse transcriptase polymerase chain reaction in lung cancer tissue was found to be more frequent in women (85%) than in men (15%).[90] However, no significant difference in ERβ expression was noted based on sex. The expression of both of these receptors in the lung cancer tissue specimens from women was more frequent than in adjacent normal lung tissue, whereas no such differences have been observed in men. However, the proliferative response to estrogen binding in NSCLC cell lines is probably mediated through ERβ.[91] The metabolic products of estrogen (catechol estrogens) could interact with DNA directly forming DNA adducts, resulting in critical mutations leading to carcinogenesis.[92] Variation in the metabolic pathways involved in the estrogen metabolism may also play a role in susceptibility to develop lung cancer. The detection of ERs and the description of carcinogenesis mechanisms involving estrogen raise the question of whether estrogen replacement therapy (ERT) is a risk factor for lung cancer. A case-control study that included smokers and never smokers reported increased risk for development of adenocarcinoma of the lung with ERT (OR, 1.7; 95% CI, 1.0 to 2.5). However, in the subset of never smokers, ERT was not associated with increased risk.[93] Other studies have failed to confirm an association between ERT and increased susceptibility to development of lung cancer.[94,95] Blackman et al[94] reported no increased risk for developing lung cancer from ERT (OR, 1.0; 95% CI, 0.8 to 1.4). There was a two-fold increased risk that was not statistically significant for adenocarcinoma in never smokers, but the sample size (n = 6) was small. In contrast, Schabath et al[95] found ERT to exert a protective effect on women from developing lung cancer, with an overall risk reduction of 35%. Thus, there is no clear evidence that suggests a causal role for estrogen in the development of LCINS at the present time. However, the increased expression of ERs in tumor tissue from women and the cell proliferative effects of estrogen require further investigation to elucidate their role in the etiopathogenesis of LCINS.

Pre-Existing Lung Disease

Pre-existing lung diseases, such as chronic obstructive pulmonary disease, pneumonia, asthma, and idiopathic pulmonary fibrosis, have been studied as potential risk factors for lung cancer.[96-100] However, patients with severe chronic obstructive pulmonary disease have also had substantial exposure to tobacco smoking. Women never smokers and former smokers with a history of benign lung disease (asthma, chronic bronchitis, emphysema, pneumonia, and tuberculosis) seem to be at increased risk for developing lung cancer.[101,102] Several epidemiologic studies have detected an association between

lung cancer and idiopathic pulmonary fibrosis.[103-105] A cohort study with an age-matched population detected an increased incidence rate ratio of 7.31(95% CI, 4.47 to 11.93; $P < .001$), and the ratio was significant even after adjusting for smoking.[106] However, another large study did not detect an increased risk for lung cancer in patients with idiopathic pulmonary fibrosis compared with the general population.[107] It is likely that the presence of chronic inflammatory lung disease confers a slightly higher risk for development of lung cancer even in the absence of additional risk factors such as tobacco smoking. However, the vast majority of patients with LCINS do not have a history of active interstitial lung disease.

Oncogenic Viruses: Human Papillomavirus

Human papillomavirus (HPV) is known to be associated with squamous cell carcinoma of the cervix, skin, esophagus, and upper airways, raising the possibility of HPV playing a role in the development of lung cancer.[108] Morphologic changes, similar to those seen in condyloma, suggestive of HPV involvement were reported in the bronchial specimens of 36 patients in a sample of 104 patients with pulmonary squamous cell cancer.[109] In Taiwanese patients with lung cancer, HPV 16/18 DNA was detected in 77 (54.6%) of 141 tumor samples.[110] The detection rate was significantly higher in LCINS than smokers with lung cancer ($P = .000005$). However, HPV DNA was detected only in two of 34 specimens from white patients with squamous cell cancer of the lung by PCR technique.[111] Reviews of published literature on HPV support the higher incidence of HPV DNA in lung cancer patients of Asian ethnicity, with incidence ranging from 9% to 42% of lung cancer tissues analyzed.[112,113]

HPV serotypes 16 and 18 are associated with lung cancer more than any other serotypes. It is suggested that these viruses are acquired through the oral cavity by intrauterine (prenatal) transmission or sexual transmission and subsequently transferred to larynx and bronchial epithelium.[113] HPV-18 *E6* and *E7* oncogenes have been shown to immortalize human tracheal epithelial cells, which are highly prone to further genetic damage.[114,115] The same effect of cell immortalization has been reported with HPV-16 *E6* and *E7* oncogenes in at least two other studies.[116-118] Jaagsiekte or ovine pulmonary adenomatosis is a neoplastic disease in sheep caused by a retrovirus. This disease is pathologically similar to human bronchioloalveolar carcinoma; however, there is not enough evidence to link these two diseases and the involvement of viruses in the development of bronchioloalveolar carcinoma.[119]

MOLECULAR CHANGES AND PATHOGENESIS OF LCINS

Carcinogenesis is a stepwise process characterized by accumulation of mutations ultimately resulting in invasive malignancy best exemplified in adenoma-carcinoma sequence in colon cancer.[120] Several molecular changes have been described in tobacco-related lung cancer and LCINS. Some of these changes have been reported to be unique to LCINS. They include chromosomal abnormalities, activation of oncogenes, inactivation of tumor suppressor genes, and mutations of genes involved in DNA repair. Gene expression profiles of adenocarcinoma of the lung and normal lung tissues from never smokers have been compared with the same tissues from patients with a history of tobacco smoking.[121] The reportedly normal lung tissue adjacent to the tumors in smokers had a gene expression pattern similar to that seen in tumors, whereas such changes were not seen in the seemingly normal lung tissue adjacent to cancer in patients with no history of tobacco smoking. These changes are hardly surprising given the known effect of tobacco on field carcinogenesis. In addition, genetic changes specific to LCINS were also described. These were decreased expression of genes associated with transforming growth factor beta signaling and changes in cell and matrix genes, suggesting different pathways in LCINS and smokers with lung cancer. However, these findings should be interpreted with caution because this study examined tissues from only 12 patients.

Chromosomal Aberrations

One of the most common chromosomal aberrations associated with lung cancer is the loss of heterozygozity in chromosome 3p.[122] In a small study, DNA gain at 16p was noted in 19 (59%) of 32 LCINS tumor samples but in only one of 10 tumor samples from tobacco-associated lung cancer patients.[123] Earlier studies (smoking status unknown) reported changes in 16p only infrequently (< 5%).[124,125] Chromosomal aberrations seen in lung cancer caused by smoking are also described in LCINS, and the presence of common aberrations possibly indicates a separate but overlapping carcinogenesis pathway.[126,127]

p53 *Mutations*

The tumor suppressor gene *p53*, which is located on 17p13, encodes a protein that plays a pivotal role in cell cycle regulation.[128] Mutations involving *p53* have been reported in 70% of small-cell lung carcinoma patients and 50% of NSCLC patients.[129] Several studies have detected hot spots on the *p53* gene, with G:C to T:A transversions being a characteristic finding in tobacco-associated lung cancer.[130-133] The incidence for G:C to T:A transversions in the lung cancer tissues from never smokers is significantly lower than that of smokers. The ratio of G:C to T:A (G→T) transversions to G:C to A:T (G→A) transitions was 1.0 in smokers and 0.34 in never smokers. Further analysis reported that this difference between never smokers and smokers was detectable only in women and not in men. These findings suggest that the *p53* mutations in LCINS are distinct from those seen in tobacco-induced lung cancer.[133] In addition, there is evidence to suggest that there may be a difference in the type of *p53* mutations between LCINS and smokers with lung cancer. The *p53* mutations in women never smokers with adenocarcinoma were predominantly transitions (83%). However, in smokers, the mutations were predominantly transversions (60%) and deletions (20%) in one study.[134]

EGFR-TK Mutations

Activation of the EGFR pathway in response to ligand binding plays an important role in cell proliferation, apoptosis, angiogenesis, and invasion.[135] Specific activating mutations in the EGFR-TK binding domain are associated with dramatic and durable benefit with EGFR-TK inhibitors such as gefitinib.[136,137] These activating mutations are present more frequently in LCINS compared with tobacco-associated lung cancer (Table 2). The reason for the high incidence of EGFR-TK mutations in never smokers is currently unknown.[138,139,147] Analysis of EGFR-TK domains in NSCLC tumor samples and normal lung tissue by PCR technique identified these mutations exclusively in NSCLC samples.[138]

K-ras *Mutations*

K-ras mutations are reported to occur in 30% to 50% of lung adenocarcinomas.[148-151] G to T transversion in *K-ras* was seen exclusively in patients with tobacco-associated adenocarcinoma of the lung compared with LCINS (43% *v* 0%, respectively; $P = .001$).[150] *K-ras*

Table 2. Distribution of EGFR Mutations in Never Smokers Versus Smokers With Lung Cancer

Study	LCINS		Tobacco-Associated Lung Cancer Patients	
	No.	%	No.	%
Shigematsu et al[138]	85	51	35	10
Pao and Miller[135]	7	47	4	5
Sonobe et al[139]				
Never smokers	44	83	—	—
Former smokers	—	—	11	50
Current smokers	—	—	5	15.2
Kosaka et al[140]	76	66	34	22
Huang et al[141]	33	58	5	42
Tomizawa et al[142]	20	47	9	13
Pan et al[143]	7	42	3	8.6
Shih et al[144]	23	56	6	29
Hsieh et al[145]	14	66	3	21
Tokumo et al[146]	25	69	13	15

Abbreviations: EGFR, epidermal growth factor receptor; LCINS, lung cancer in never smokers.

Table 3. Molecular Characteristics of LCINS and Tobacco-Related Lung Cancer Patients

Molecular Markers	LCINS	Tobacco-Associated Lung Cancer
Chromosomal aberrations		
16p DNA gain	Common, 59%	Very rare, < 5%
Gene mutations		
p53 G→T to G→A transversions*	Low, ratio = 0.23	High, ratio = 1.5
p53 transition mutations*	Very common, 83%	Rare, 20%
K-ras	Very rare, 0%-7%	Common, 30%-43%
EGFR-TK	Common	Rare
Epigenetic changes		
p16 and *APC* methylation rate	Low	High
Hypermethylation of *hMLH1*†	Common	Rare
Hypermethylation of *hMSH2*†	Common	Rare

Abbreviations: LCINS, lung cancer in never smokers; EGFR-TK, epidermal growth factor receptor tyrosine kinase.
*Data from women with LCINS
†Loss of protein expression in mismatch repair genes.

mutation is seen predominantly in smokers with lung cancer.[134,152] The *EGFR-TK* and *K-ras* mutations seem to be mutually exclusive.[4]

DNA Methylation

Epigenetic changes (such as DNA methylation), well-described in lung cancer, differ from gene mutations because they are potentially reversible.[153,154] Epigenetic changes reported in LCINS differ from those described in lung cancer associated with tobacco smoking.[155] Methylation of the cytosine in the CpG islands in the promoter region results in gene silencing. The methylation index (number of genes methylated/number of genes tested), which reflects the overall methylation status, is greater in tobacco-associated lung cancer compared with LCINS.[155] More specifically, smokers with lung cancer had a higher methylation rate in *p16* and *APC* than LCINS, and the rates were dependent on the amount of exposure to tobacco smoke.[155-157] The increased methylation rate of *p16* in tobacco-associated lung cancer compared with LCINS has been reported by others as well[158]; however, this study did not detect a difference in the methylation of *DAPK* and *RASSF1A* between the two groups. In another study, loss of protein expression of mismatch repair genes *hMLH1* and *hMSH2* was significantly higher in never smokers with NSCLC compared with patients with tobacco-associated NSCLC (*hMLHI*, 70% and 46%, respectively; *hMSH2*, 40% and 10%, respectively).[159] Promoter hypermethylation seems to be the principal cause for loss of protein expression in *hMLH1* and *hMSH2* genes. Table 3 lists some of the unique genetic markers known in LCINS.

Lung cancer as a result of tobacco smoking is a complex disease with many unique genetic features, and LCINS shares some of these features. However, there are differences in the genetic makeup of these two diseases. This is attributed to the difference in tobacco smoke exposure. The absence of mainstream tobacco smoke results in variations in *p53* mutational spectrum, absence of *K-ras* mutations, and differences in the frequency of gene inactivation by hypermethylation in LCINS. However, other features, such as increased frequency of DNA gain at 16p, promoter methylation of *hMLH1* and *hMSH2* mismatch repair genes, and EGFR-TK mutations, are unexplained in patients with LCINS. These differences are significant and suggest possible multiple etiologies including inherited genetic susceptibility and environmental, hormonal, and other unknown factors. Further investigation is required to clarify and outline the role of each of these factors in LCINS carcinogenesis. In addition, these unique molecular changes, particularly the mutual exclusivity of *K-ras* and *EGFR-TK* mutations, indicate that LCINS is a distinct molecular entity that may share some overlapping pathways with the more common tobacco-associated lung cancer.[4,160] Better understanding of the molecular mechanisms underlying this disease would undoubtedly improve the outcomes of patients with LCINS and perhaps even patients with tobacco-associated lung cancer.

CLINICAL PRESENTATION

The clinical presentation of LCINS is distinct compared with tobacco-associated lung cancer with regard to sex and histology. LCINS affects women disproportionately more than men, with nearly half the women with lung cancer globally being lifelong never smokers.[3] The most common histologic subtype is adenocarcinoma, which accounts for nearly 60% of LCINS (Table 1). It is unclear whether the stage at the time of initial presentation is different between LCINS and tobacco-associated lung cancer. The stage at presentation was stage IV in 70.9% of LCINS patients compared with 56.1% of patients with tobacco-related NSCLC.[15] In a group of patients with adenocarcinoma of the lung, no such difference was reported between never smokers and smokers.[161]

Response to Systemic Therapy

There are only limited data available on response to chemotherapy in LCINS. In a small retrospective study, no differences in response rates were seen between smokers and never smokers with advanced NSCLC receiving a wide variety of cytotoxic chemotherapy regimens.[7] Data from the Tarceva Responses in Conjunction

Table 4. Survival of Smokers and Never Smokers With Lung Cancer on EGFR-TK Inhibitors

Regimen	Study	Survival			
		LCINS		TALC	
		Hazard Ratio	95% CI	Hazard Ratio	95% CI
Gefitinib monotherapy	Thatcher et al[165]	0.67	0.49 to 0.92	0.92	0.79 to 1.06
Erlotinib monotherapy	Shepherd et al[164]	0.4	0.3 to 0.6	0.9	0.7 to 1.0

Abbreviations: EGFR-TK, epidermal growth factor receptor tyrosine kinase; LCINS, lung cancer in never smokers; TALC, tobacco-associated lung cancer.

with Paclitaxel and Carboplatin (TRIBUTE) trial also found no significant difference in median overall survival between LCINS and tobacco-associated lung cancer patients with cytotoxic chemotherapy alone (10.1 months for LCINS, 9.1 months for current smokers and 10.9 months for former smokers).[162]

Dramatic differences in response to therapy have been reported with the use of EGFR-TK inhibitors in LCINS compared with tobacco-related lung cancer (Table 4). LCINS patients had higher response rates to gefitinib compared with tobacco-related lung cancer patients (36% *v* 8%, respectively; $P < .001$).[163] Similar results have been reported with erlotinib as well, with significantly higher response rates in never smokers compared with patients with a history of tobacco smoking (24.7% *v* 3.9%, respectively; $P < .001$).[164] This large prospective study, which randomly assigned patients with advanced NSCLC to either erlotinib or placebo, also reported better survival in never smokers treated with erlotinib compared with current or former smokers. In the Iressa Survival Evaluation in Lung Cancer trial, gefitinib did not improve survival when compared with placebo in an unselected group of patients with advanced NSCLC who experienced progression after platinum-based therapy.[165] Although the overall survival was disappointing, subgroup analysis identified improved survival in never smokers treated with gefitinib compared with placebo (hazard ratio, 0.67; 95% CI, 0.49 to 0.92; $P = .012$; median survival time, 8.9 *v* 6.1 months, respectively). The prospective TRIBUTE study randomly assigned patients with previously untreated advanced NSCLC to receive either paclitaxel, carboplatin, and placebo or paclitaxel, carboplatin, and erlotinib followed by maintenance therapy (placebo or erlotinib).[162,166] In the subset analysis of never smokers with unresectable advanced NSCLC, the group treated with erlotinib had an impressive median survival time of 22.5 months compared with only 10.1 months for patients assigned to receive placebo (hazard ratio, 0.49; 95% CI, 0.28 to 0.85). Studies are ongoing to define the role of EGFR-TK inhibitors (as single agents and in combination with chemotherapy) in previously untreated lifelong never smokers or light smokers with advanced NSCLC.

Survival

Nordquist et al[161] reported better survival in never smokers (n = 132) compared with current smokers (n = 522) with adenocarcinoma of lung, with a 5-year survival rate of 23% for never smokers compared with 16% for current smokers ($P = .004$). A recent study from Utah reported better survival in LCINS patients with advanced-stage disease.[15] This study consisted of 180 LCINS patients and 1,040 ever smokers with lung cancer. Staging was divided into the following three groups: local, regional, and distant. The 5-year survival rates for local stage LCINS and tobacco-related NSCLC were 40.5% and 69.8%, respectively; the overall 3-year survival rates for regional stage disease were 34.2% and 22.6%, respectively; and the overall 3-years survival rates for all stages were 9.3% and 3.2%, respectively. A retrospective study from Singapore reported no difference in response to treatment between LCINS and tobacco-associated lung cancer patients.[7] Of the 317 patients included in this study, 117 (36%) were never smokers. The median survival times for never smokers and smokers were 18.5 and 13.6 months, respectively, and the difference was not statistically significant. The majority of the patients in this study had advanced disease. In the tobacco-related NSCLC group, 86.4% of patients had stage III or IV disease; in the LCINS group, 89.5% of patients had stage III or IV disease. The influence of comorbidities, which are so often present in patients with a long history of tobacco smoking, on survival could not be discounted in these retrospective studies.

FUTURE DIRECTIONS

Current evidence indicates that LCINS is a distinct disease entity with unique molecular and biologic characteristics. There are striking differences in the incidence of LCINS across the globe. These variations are likely to be a result of the difference in exposure to some known and some yet unidentified carcinogens. The higher incidence of LCINS and EGFR-TK mutations in patients of East Asian ethnicity is particularly noteworthy and suggests a possible role of inherited genetic factors. Dramatic results seen with the use of EGFR-TK inhibitors has focused the much-needed spotlight on this distinct disease. Although LCINS shares some molecular features typically seen with tobacco-related lung cancer, the presence of unique genetic and epigenetic markers suggests that a separate but overlapping carcinogenesis pathway leads to LCINS. With improved understanding of the molecular biology of LCINS, it is likely that LCINS will be treated very differently than lung cancer associated with tobacco smoking. Of course, tobacco smoking continues to be the main cause of lung cancer, and every effort should be made to discourage adolescents from starting tobacco smoking and to promote smoking cessation vigorously in adults.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest.

No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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