Teen Smoking, Field Cancerization, and a "Critical Period" Hypothesis for Lung Cancer Susceptibility

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Cigarette smoking by children and adolescents continues to be prevalent, and this fact represents a major public health problem and challenge. Epidemiologic work has previously suggested that exposure of the lung to tobacco carcinogens at an early age may be an independent risk factor for lung cancer. Recent studies at the molecular and cellular levels are consistent with this, now suggesting that early exposure enhances DNA damage and is associated with the induction of DNA alterations in specific chromosomal regions. In this paper we hypothesize that adolescence, which is known to be the period of greatest development for the lung, may constitute a "critical period" in which tobacco carcinogens can induce fields of genetic alterations that make the early smoker more susceptible to the damaging effects of continued smoking. The fact that lung development differs by sex might also contribute to apparent gender differences in lung cancer susceptibility. Because this hypothesis has important implications for health policy and tobacco control, additional resources need to be devoted to its further evaluation. Targeted intervention in adolescent smoking may yield even greater reductions in lung cancer occurrence than otherwise anticipated. *Key words*: **cigarette smoking, field cancerization, lung cancer, susceptibility, tobacco.** *Environ Health Perspect* **110:555–558 (2002). [Online 12 April 2002]**
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Cigarette smoking by adolescents is arguably one of the most important public health problems of our time. The percentage of U.S. high school students who smoke rose by nearly one-third between 1991 and 1997 (*1*), although it has decreased somewhat since then. A prospective 3-year survey of 401 U.S. children also found that 217 (54%) tried smoking in grades 5–7 (10–13 years of age). In this study, 63 students smoked throughout the 3 years of the study, with 90 trying smoking in the fifth or sixth grade but stopping by seventh grade, and 64 starting in the last year of the study (seventh grade) (*2*). Recent work now indicates the clinical effects of these trends in smoking initiation: national cancer surveillance data show a birth cohort pattern of lung cancer mortality after 1950 that reflects the impact of teenage cigarette smoking in people younger than 45 years of age (*3*).

A feature of early smoking that is only now emerging from basic molecular research is the relationship between tobacco-induced changes in the constitution of lung cells and their subsequent increased susceptibility to malignant transformation. Smoking-related genetic alterations of the respiratory epithelium occurring in early life may be responsible for the epidemiologic observations which suggest that early smoking initiation confers an increased lung cancer risk independent of years of smoking duration or smoking intensity (*4–7*). Gender-related differences in lung development may also be linked to epidemiologic observations that women and men differ in lung cancer risk at a given level of smoking (*8,9*). In this paper, we propose that normal developmental processes, in concert with exposure to tobacco smoke, may promote an abnormal clonal proliferation initiating a process termed "field cancerization." Abnormal clonal fields caused by early smoking may become the fertile ground for the emergence of potentially lethal cancers later in life.

Human Lung Development

The human lung and airways are known to undergo a complex development. At birth the lung contains somewhere between 17 and 70 million alveoli (*10*). By 7 years of age, a 13-fold increase in lung volume has taken place (*11*), and in adult lungs, individual alveoli number some 200–600 million. The rate of lung growth, measured by volume, is thought to be linear from birth to approximately 11–12 years of age (with females having a slightly earlier end to the rapid, linear phase of growth in volume) (*12*). The onset of puberty coincides with dramatic changes in lung function. Sudden increases in spirometric lung function at the pubertal growth spurt have been recorded for each sex (*13*). Airways in females may be larger than in males before puberty (*11*), but during and after puberty airway size relative to lung size in boys increases beyond that in girls (*14*). Hence, growth of airways and parenchyma appears to be proportional in females, but in males large airway growth lags behind growth of the parenchyma (*11*). Tager et al. (*12*) have shown that males reach a plateau in lung volume sometime after 24 years of age (slow, continued growth may persist through the early 30s), whereas females reach their maximum growth earlier, at approximately 18 years of age.

Cigarette smoking in adolescence exacerbates respiratory ailments and also alters the growth of lung volume. The linear phase of growth in lung volume is approximately 1 year shorter in males who smoke compared with those who never smoked and 2–3 years shorter in female smokers (*12*). Gold et al. (*15*) further showed that the plateau level in lung volume reached by smokers is less than that of nonsmokers. These data show that tobacco smoking diminishes the rate of growth of the lungs when exposure occurs before the age at which growth plateaus (18 years of age in females and approximately 24 years of age in males). The mechanism responsible for this drop in the rate of growth is unknown; however, tobacco-related killing of dividing cells and their replacement through a process analogous to wound healing is almost certain to take place in the airways and throughout the lung. Amid this interplay of developmentally programmed cell proliferation, genotoxic exposure and wound healing may give rise to the conditions for the pathologic process termed "cancerization of cellular fields."

Field Cancerization

Slaughter et al. (*16*) first articulated the explicit concept of "field cancerization" in 1953 based on observations of the occurrence of multiple primary tumors in the same tissue in proximity to each other. Slaughter et al. (*16*) reported that grossly normal epithelium can be "preconditioned"

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by carcinogen exposure; this is consistent with the hypothesis that early tobacco-induced somatic mutations are propagated by enhanced cell replication into a clonal "field" of genetically altered progenitor cells. Recent work has shown that large clones of somatically altered cells (e.g., 90,000 cells) can be found in grossly normal lung tissue after resection for lung cancer or after biopsy of normal tissue in the airways of smokers (*17,18*). In fact, it has been repeatedly shown that fields of cells containing loss of heterozygosity (deletion of one copy of allelic DNA sequences) within the chromosome 3p21.3 region are among the earliest and most frequent events found in normal-appearing tissues in the lungs of smokers and in the lung cancers of smokers (particularly for the squamous histology) (*17–20*). This loss is frequent among clonal outgrowths and has been observed in the lungs of very young smokers with normal airways (*17*). Furthermore, Franklin et al. (*21*) showed that large patches of cells (extending into the lower lobe bronchi of both lungs) within grossly normal-appearing lung epithelium can contain a single somatically arising *p53* mutation, thus strongly implicating a developmentally early clonal process. Taken together, these observations demonstrate that the induction by tobacco carcinogens of fields of somatically altered cells is a frequent and perhaps ordered feature of lung cancer. Interestingly, carcinogen exposure that occurs at a young age is hypothesized to be an independent cancer risk factor for tumors arising in other tissues displaying field cancerization; cutaneous exposure to ultraviolet light in children is known to independently enhance risk of melanoma in adulthood (*22–24*), and ionizing radiation exposures during puberty (from Hiroshima and Nagasaki atomic bomb explosions) enhanced breast cancer risk in Japanese (*25*). Hence, tobacco carcinogen exposure of growing lungs could quite plausibly have a far greater likelihood of producing larger, more biologically significant clonal expansions compared with exposures that occur after lung growth has slowed or ceased.

The links between these somatic alterations in the lung and early smoking initiation have come very recently. We (*26,27*) and others (*28*) have observed associations of tobacco-related DNA damage and mutational changes with self-reported age at smoking initiation in patients with lung cancer. Most significantly, younger ages of smoking initiation were associated with a higher prevalence of chromosomal deletions within the specific genomic region implicated in field cancerization in the lung (i.e., 3p21). The highest prevalence of 3p21 loss occurred in lung cancer patients who reported smoking before 16.5 years of age (*27*). By this time in life, advanced Tanner pubertal stages are recorded in almost all persons and most boys and girls will have undergone their maximal growth spurt. These associations lead to a novel hypothesis: Growth and development of the lungs during adolescence may set up a critical period of susceptibility to tobacco-related DNA damage. Because lung development proceeds through the teen years, exposure to tobacco carcinogens during this period may lead to the propagation of fields of altered epithelial cells, particularly in the airway, that later evolve into frank malignancy. Exposure after the critical growth period may have a much lower probability of inducing large, clonal outgrowths with the same malignant potential. The elements of this developmental hypothesis are summarized in Table 1. That these mechanisms operate to create a critical period of tobacco susceptibility in adolescence, however, is only a hypothesis. Ours were cross-sectional observations based on self-reported age of smoking initiation among persons who developed lung cancer and should be interpreted with caution until confirmed.

The Future

Traditional epidemiologic methods have provided limited insight into the important question of age at smoking initiation and lung cancer risk. A younger age of starting to smoke, by increasing duration of smoking at any particular age, is, of course, predicted to increase lung cancer risk. There is limited epidemiologic evidence that age at onset of smoking independently confers an increase in cancer risk (*29,30*). It has been very difficult to disentangle the effects of age at smoking initiation from those of smoking duration and intensity, especially among current smokers. With the growing population of former smokers, however, this question can now be revisited with better prospects for identifying age-specific risks. Also, early smoking may affect specific subtypes of lung cancer. Much of the molecular evidence supporting early field cancerization is drawn from studies of the proximal airways accessible to the bronchoscope where squamous cell carcinomas most frequently arise. Less evidence is available to address this process in other cell types in the periphery of the lung. If subtype specificity does exist, the independent effect of early smoking on lung cancer would be considerably more difficult to detect in the many epidemiologic studies that have grouped different histologies together. In addition, because adenocarcinoma is becoming increasingly prevalent, this may produce conflicting results between older and more recent studies of this question, absent adjustment for histologic subtype of cancer. Furthermore, early smoking may be associated with competing risks that could obscure the effects of smoking initiation on lung cancer risks. For example, in the Nurses Health Study, women who started smoking before 15 years of age experienced significantly greater risks for cardiovascular mortality compared with women who started smoking later in life (*31*).

In the future, how can we bring into focus the unique physiologic and toxicologic dimensions of youth smoking and lung cancer so that we can judge their real clinical and public health importance? One way is to continue to investigate the molecular epidemiology of lung cancer and reinforce the links between cancerous somatic mutations and early smoking. This requires the standardized collection of detailed smoking histories from lung cancer patients and their integration with molecular information derived from high-density genetic maps of chromosomal loss occurring within patients' lung tumors. The search for progressively more precise physical mapping of the regions of chromosome 3p21 associated with early smoking initiation is an extension of ongoing somatic linkage analyses to identify putative lung tumor suppressor genes using lung cancer cell

Table 1. Evidence supporting a critical period for the induction of lung cancer.

Type of evidence	Description of evidence	Reference
Epidemiologic	Age at onset of cigarette smoking is a risk factor for lung cancer	(*4–7*)
	Women may be at higher risk compared with men	(*8,9*)
Developmental	Lung volumes increase significantly during the adolescent growth spurt	(*11,13*)
	Respiratory airways in girls mature faster compared with boys	(*11,12,14*)
Cellular	Fields of genetically altered cells are induced by exposure to tobacco smoke	(*16–20*)
	Clonal expansion of abnormal fields may be augmented by developmentally programmed cell proliferation	(*22–25*)
Molecular	The earliest genetic changes in lung cancer occur in highly fragile chromosomal sites, including the 3p21 region	(*17,18*)
	Deletions of 3p21 occurred most frequently in lung cancers from persons who started smoking early in adolescence	(*27*)
	DNA damage within the lung is increased in smokers who started smoking early in adolescence	(*26,28*)

lines (*32*). The possibility that a heightened sensitivity to chromosomal loss may accompany exposures around puberty serves to emphasize the importance of these investigations. At least 25 gene candidates have been identified within the 630-kb homozygous deletion region that is thought to harbor one or more genes critical in lung cancer. In the present context, however, it must be emphasized that the linkage under scrutiny is not cancer per se but cancer arising after a developmentally specific exposure to tobacco carcinogens. Hence, we have no *a priori* reason to suppose that the ongoing search within the 3p region for lung cancer suppressor genes and the search for genes conferring increased risk by smoking at an early age will converge on the same gene(s). Interestingly, the 3p21 marker we found associated with early smoking in our studies (D3S1478) maps to a location about 1.5 Mb telomeric to the aforementioned commonly deleted region. Finally, we must add that, aside from the specific genes involved in the critical early stages of lung cancer, we still do not understand the basis for the intrinsic chromosomal instability of the 3p region. The fragility of the region contributes to many cancers in addition to lung cancer. Curiously, in any one patient, this instability affects one parental allele preferentially, a phenomenon referred to as allele-specific mutation (*17*).

We need clinical and population scientists to clarify how researchers will refine the assessment of lung development on the one hand and more precisely quantify early smoking initiation on the other. Our understanding of adolescent lung development is based on an extensive body of functional studies. This must be contrasted with the principal mechanism driving the process of field cancerization, which is most likely increased cell proliferation within a relatively small progenitor epithelial cell compartment. Clearly, we need more research documenting the cellular correlates of spirometric measures of growth in lung function and addressing both unexposed and tobacco-exposed conditions.

As for smoking exposure assessments, it is critical to recognize that multiple patterns of smoking initiation occur (*2*). We often assume that smoking initiation occurs in uniform stages; initiation involves an early experimentation stage characterized by intermittent and low tobacco exposures; subsequently, a period of more regular cigarette consumption ensues, and later a "ramping up" of daily consumption may take place. What are the most significant parameters to focus on that will yield the most sensitive measure of a heightened developmental susceptibility to tobacco damage? Studies by Gold et al. (*15*) indicated that smoking five or more cigarettes per day was associated with decrements in lung function in boys and girls. In our studies, we took the age of smoking initiation to be the age at which patients reported daily smoking of any number of cigarettes. Also, because the physiologic effects of puberty must be addressed, the fact that the age of onset of puberty is highly variable among individuals requires even further scrutiny of our exposure measurement tools. The timing of puberty varies by sex and perhaps ethnicity, and it may be undergoing long-term secular change. The foregoing challenges illustrate that new instruments must be developed and validated before the most optimal tests of the existence of a "critical period" for susceptibility to lung cancer can take place.

Examination of the hypothesis put forth here also may have implications for tobacco control policy. The consequences of increasing our knowledge in addressing these now-critical questions are clear; if exposure to tobacco smoke before the lung is developmentally mature represents a "critical period" resulting in enhanced, independent cancer risk, stopping cigarette smoking in adolescents takes on vital new importance. Those involved in the crucial area of tobacco-associated cancer prevention should be encouraged by the knowledge that delaying teen smoking by even 1–2 years may result in an important reduction in lung cancer risk. Programs aimed at early smoking prevention should be encouraged because they will clearly reap real rewards.

Furthermore, in the current environment of renewed attention to tobacco-associated cancer prevention, we should appreciate that knowledge of this sort is crucial to public health. Now is the time to turn the tools of the laboratory and the epidemiologist more generally on this question and others like it. These tools are a vital part of the prevention enterprise and must be recognized as such by the public health community and basic and applied scientists. Ultimately, we share the common goal of understanding this disease and using this knowledge to benefit all of us.

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