# Environmental tobacco smoke, carcinogenesis, and angiogenesis: A double whammy?

**In this issue of *Cancer Cell*, Zhu and coworkers demonstrate that environmental tobacco smoke (ETS) results in tumor angiogenesis, and provide evidence that the responsible factor in ETS is nicotine. While a weak carcinogen, nicotine promotes carcinogenesis by a number of different mechanisms. The authors' findings provide further evidence of the harmful effects of ETS and indicate the desirability of reducing exposure to it.**

Included among the accepted medical dogmas are: (1) exposure to tobacco smoke, either directly or indirectly, is injurious to health; and (2) new blood vessel formation (neoangiogenesis) is essential for tumor growth. In this issue of *Cancer Cell*, Zhu and coworkers demonstrate a link between these two phenomena (Zhou et al., 2003). Before we discuss how they demonstrated the connection and the significance of their findings, some background information needs to be reviewed.

Usage of tobacco products results in many health hazards, including cardiovascular disease, nonmalignant respiratory disease, and cancer. Smokers have increased rates of many cancers, especially those arising in the lung, head and neck, bladder, and cervix. More than 4000 compounds have been identified in cigarette smoke, more than 60 of which are known carcinogens and are present both in the particulate and vapor phases of cigarette smoke (Hecht, 2002). The major carcinogens include the polycyclic aromatic hydrocarbons and N-nitrosamines. Cigarette smoke may be inhaled directly by smokers (mainstream smoke), released from the burning end of cigarettes (sidestream smoke), or exhaled by smokers. The latter two forms constitute environmental tobacco smoke (ETS, or second hand smoke) that affects all individuals in the vicinity of smokers. ETS is harmful, although dilution by environmental air makes it less dangerous than mainstream smoke. While the US is among the leading nations in the stringency of its laws banning smoking in public places, cotinine, a metabolic product of nicotine, can be detected in the serum of most nonsmokers, indicating widespread exposure to ETS (Brownson et al., 2002). While the increased cancer risks of ETS are modest, the total weight of the evidence is that it is a human carcinogen responsible for an estimated 3000 lung cancer deaths in never-smokers (Brownson et al., 2002).

The tobacco engine is powered by nicotine, and cigarettes have been referred to as nicotine delivery devices. Nicotine, while a weak carcinogen, is one of the most addictive substances known. The concentration of nicotine in sidestream smoke is greater than its concentration in mainstream smoke. Nicotine exerts its cognitive and addictive properties by interacting with neuronal nicotinic acetylcholine receptors that are widely distributed in the brain (Itier and Bertrand, 2001; Leonard and Bertrand, 2001). However, nicotinic receptors are also expressed in other tissues, including vascular endothelium (Conklin et al., 2002; Heeschen et al., 2003) and bronchial epithelium (Minna, 2003; West et al., 2003). Thus, nicotine may promote carcinogenesis by indirect mechanisms, including stimulation of angiogenesis (Heeschen et al., 2003), and by activating the Akt signaling pathway in bronchial epithelial cells (Minna, 2003; West et al., 2003). Nicotine increases oxidative stress, activates NF-κB, induces apoptosis, and sensitizes cells to genotoxic and xenobiotic stresses (Crowley-Weber et al., 2003). Additional potential roles of nicotine in carcinogenesis have been discussed by Minna (2003).

The pioneering work by Folkman and others has demonstrated that tumors require neoangiogenesis (new blood vessel formation) for growth, and several clinical trials targeting tumor vasculature are being conducted currently (Dredge et al., 2003). The structure and composition of blood vessels and lymphatics vary with their size, type, and location, but all vessels are lined by endothelial cells. Endothelial cells may arise from existing differentiated cells or from circulating endothelial cell precursors derived from the bone marrow. Many molecules, secreted both by malignant and nonmalignant cells, stimulate angiogenesis. Neoangiogenesis commences early during the multistage pathogenesis of lung cancer, and angiogenic lesions are frequent in the bronchi of smokers (Keith et al., 2000). Nicotine, at concentrations found in the plasma of smokers, stimulates the proliferation of endothelial cells acting via nicotinic acetylcholine receptors, and, possibly, by other mechanisms (Jain, 2001). In addition, nicotine stimulates atheroma formation and the growth of lung cancers (Jain, 2001).

Because nicotine is a major component of ETS, Zhou and coworkers postulated that ETS would accelerate tumor angiogenesis (Zhou et al., 2003). They demonstrate that exposure to ETS at levels present in smoking environments stimulated growth of a murine tumor model, enhanced tumor vessel density, and increased growth factors and circulating endothelial cell precursors. To further prove that the oncogenic effects of ETS were mediated, at least in part, by the angiogenic effects of nicotine, they demonstrated that the effects were reduced by mecamylamine, a nicotine receptor antagonist, and by statins, compounds that interfere with the formation of endothelial precursor cells. As tobacco smoke contains several thousands of compounds, the possibility that substances in ETS other than nicotine contribute toward neoangiogenesis should be considered.

What is the significance of these findings? While the effects of nicotine on neoangiogenesis have been known for a few years, these observations have now been extended to ETS. Although smoking is the major cause of lung and some other cancers, about 15% of lung cancers arise in lifetime never-smokers. ETS is believed to be an important factor in the causation of these tumors. Clearly, ETS is harmful, and is associated with diseases other than cancer. One potentially reassuring finding is the observation that statins, drugs that are widely used as cholesterol-lowering agents, may decrease the carcinogenic effects of ETS. The findings by Zhou et al. provide further evidence that reduction of exposure to ETS is a desirable goal of major

public health importance. While smokers may have the right to smoke, nonsmokers should have the right to be protected from harm resulting from the action of smokers. Reduction of exposure to ETS should be the goal of all nations.

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Adi F. Gazdar*

Hamon Center for Therapeutic Oncology Research and Department of Pathology, UT Southwestern Medical Center, Dallas, Texas 75390
*E-mail: adi.gazdar@utsouthwestern.edu

**Selected reading**

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# Cell cycle progression without cyclin E/CDK2: Breaking down the walls of dogma

**G1 is the phase of the cell cycle wherein the cell is responsive to growth factor-dependent signals. As such, G1 regulation is frequently disrupted in cancer through deregulation of cyclin/CDK activity; deregulation of G1 phase provides tumorigenic cells with a growth advantage. Cyclin E, the regulatory cyclin for CDK2, is considered a requisite regulator of G1 progression. Cyclin E is overexpressed in cancer, suggesting that cyclin E/CDK2 deregulation contributes to tumorigenesis. Two papers now challenge both the concept that cyclin E/CDK2 is a requisite component of the cell cycle machine and efforts to develop cyclin E/CDK2 inhibitors as antiproliferative therapeutics.**

The E type cyclins and their catalytic partner, CDK2, participate in the regulation of retinoblastoma protein inactivation, establishment of the prereplication complex (pre-RC), and initiation of S phase (Figure 1); their participation in these critical regulatory steps has resulted in the assumption that both cyclin E and CDK2 are indispensable for cell cycle progression. Support for this notion was initially provided by experiments utilizing a dominant negative CDK2 molecule to demonstrate that CDK2 activity is required for cell cycle progression of certain tumor-derived cell lines (van den Heuvel and Harlow, 1993). An essential role for E/CDK2 has two critical implications. First, as an essential enzyme, loss of either component should impede cell cycle progression. Second, as unchecked cell proliferation is a hallmark of human cancer, the cyclin E/CDK2 kinase should be a logical target for the development of anticancer therapeutics. In point of fact, cyclin E is overexpressed in human breast cancer, and its overexpression correlates with poor prognosis (Keyomarsi et al., 2002). However, two papers now challenge the notion that the cyclin E/CDK2 kinase is an essential component of the cell cycle machine.

In one approach, cyclin E was eliminated from the mouse via targeting of both genes encoding E type cyclins, cyclins E1 and E2 (Geng et al., 2003), and in the second, CDK2 itself was disrupted (Ortega et al., 2003). While the phenotypes are not entirely overlapping as one might expect, they do culminate with the startling revelation that neither E type cyclins nor CDK2 are strictly required for either embryonic development or for continuous cell cycle progression.

As with elimination of another G1 cyclin, cyclin D1 (Sicinski et al., 1995), the elimination of E type cyclins resulted in focal abnormalities. Defects were observed in the development of cell types that required repeated rounds of endoreplication (repeated rounds of S phase without intervening cell division) such as trophoblast giant cells. Such a phenotype might have been anticipated from earlier examination of cyclin E function in *Drosophila* development (Sauer and Lehner, 1995). Surprisingly, CDK2 ablation did not result in apparent defects in endoreplication cycles. Defects were also observed in spermatogenesis in $E2^{-/-}$ and $E1/E2^{-/-}$ mice that resulted in eventual male sterility. $CDK2^{-/-}$ mice, like cyclin E deficient mice, also exhibited defects in male spermatogenesis. Additionally, $CDK2^{-/-}$ mice also exhibited defective female gametogenesis, implicating the cyclin E/CDK2 kinase in the regulation of meiotic cell cycles. While it is far from settled,