

Perspective*Perspective on Meireles et al., p. 707*

Early Changes in Pulmonary Gene Expression following Tobacco Exposure Shed Light on the Role of Estrogen Metabolism in Lung Carcinogenesis

Jill M. Siegfried

Abstract

This perspective on Meireles et al. (beginning on p. 707 in this issue of the journal) discusses the increasing evidence for the role of female steroid hormones in lung cancer development and progression. The novel work of Meireles et al. is the first evidence for the rapid upregulation by tobacco smoke of a key *cytochrome P450* gene that can metabolize estrogens such as β -estradiol to potentially carcinogenic catechol and quinone forms, as well as the first evidence for the colocalization of β -estradiol and estrogen receptors in murine airway epithelium. Actions of estrogens that contribute to lung carcinogenesis, especially in the presence of tobacco smoke, may involve both reactive intermediates that damage DNA and steroid hormone receptor signaling that promotes growth. *Cancer Prev Res*; 3(6): 692–5. ©2010 AACR.

The devastating disease lung cancer continues to increase in incidence around the world. It is the number one cause of cancer death for both men and women in the United States and accounts for more cancer deaths in U.S. women than do breast, ovarian, and endometrial cancers combined (1). At present there is no recommended lung cancer early detection or screening modality, and no chemopreventive agents have established activity against lung cancer development in human populations.

Increasing evidence suggests a role for the steroid hormones estrogen and progesterone in lung cancer; the predominant estrogen in females, β -estradiol, has many growth-promoting actions on malignant lung cells (2, 3), whereas progesterone seems to act as a growth-inhibiting hormone (4). Although the actions of these hormones in the lung are not completely analogous to breast cancer, many of the strategies and therapeutic agents that have been successful for breast cancer control may also have applications for lung cancer treatment and prevention.

A recent study found that the antiestrogen tamoxifen protected against a subsequent diagnosis of lung cancer among women who were treated for breast cancer (5). This finding is important because not only has the risk of endocrine-related cancer been linked to the risk of lung

cancer (6) but radiation to the chest area for breast cancer treatment increases the risk for a subsequent lung cancer (7). Furthermore, antiestrogens developed for breast cancer could have a wide application in lung cancer patients because a very high proportion (>80%) of non-small cell lung carcinomas in men and women express at least one of the estrogen receptor subtypes (8, 9) and/or the enzyme aromatase (10), which converts androgen precursors to estrogens. Antiestrogens also could have chemopreventive effects against the development of such lung tumors in high-risk individuals.

Because pulmonary estrogen effects may be largely due to local production of estrogens within lung tissue rather than to circulating estrogens, estrogens may be active in the lungs of men as well as women. In postmenopausal women, pulmonary estrogen production may continue after ovarian production ceases, and in premenopausal women, circulating estrogens could heighten procancer effects of local pulmonary estrogen. The lung cancer risk of female never smokers, who are often diagnosed at a relatively young age (<50 y old), is estimated to be about twice that of male never smokers in prospective cohort studies (11). Hormone replacement therapy (HRT) in postmenopausal women had been shown in retrospective studies published before 2000 to have some association with increased lung cancer risk, but more recent retrospective studies showed that HRT use may reduce the risk of a future lung cancer diagnosis (12, 13). In contrast, HRT use near the time of or after a lung cancer diagnosis increased lung cancer progression (14, 15). A large randomized prospective population study, the Women's Health Initiative, found in 2009 that HRT significantly increased the risk of death from lung cancer and showed a trend for more lung cancer diagnoses (15). Although the reasons for contradictory results

Author's Affiliation: University of Pittsburgh Cancer Institute, Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania

Corresponding Author: Jill M. Siegfried, Hillman Cancer Center, 5117 Centre Avenue, Suite 2.18, Pittsburgh, PA 15213. Phone: 412-623-7769; Fax: 412-623-7768; E-mail: siegfriedjm@upmc.edu.

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about lung cancer risk are not clear, HRT formulations have varied over time, and it is possible that some types of HRT use result in systemic lung cancer protective effects, such as stimulation of the immune system and downregulation of insulin-like growth factor-I by exogenous estrogens, whereas local estrogen production in the lung remains intact or is even reduced. The progesterone in HRT also might protect against lung cancer development (4) but might become less effective as lung cancer progresses. Our laboratory has documented downregulation of the progesterone receptor in lung tumors compared with matching normal lung tissue from the same patient.¹ Other factors in the retrospective risk studies, such as smaller sample sizes, variations within each study in the amount and type of HRT used, and recall bias, may also account for some of the discrepancies between the risk results of these studies. The retrospective and prospective studies agree, however, in finding that HRT use after a lung cancer diagnosis increases lung cancer progression.

An important confounder in assessing the action of estrogens in lung cancer is tobacco exposure, the major risk factor for this disease. Although there are >25,000 deaths every year in the United States from lung cancer in patients who never smoked, of whom the majority are women, the balance (~85%) of the 170,000 annual U.S. lung cancer deaths is associated with past or present tobacco use. It is well recognized that induction of phase I cytochrome P450s (CYP) by the carcinogens in tobacco smoke results in increased excretion of estrogen metabolites because these metabolites are then made more water soluble by the action of phase II enzymes. Women who smoke heavily often have reduced circulating levels of estrogens that can result in menopause-like effects, including infertility (16). This estrogen reduction might result in overall lower systemic estrogenic effects over time, but increased estrogen metabolism also could result in more conversion of estrogens in tissues to reactive intermediates such as 4-hydroxyestradiol, which could drive lung carcinogenesis if produced locally in the airways. 4-Hydroxyestradiol, which is produced mainly through the actions of CYP1B1, can be oxidized to a quinone that forms DNA adducts and is a more potent and longer-lasting activator of estrogen receptors (ER; ref. 17).

In this issue of the journal, Meireles et al. examined early changes in gene expression following tobacco exposure in lung tissue in a murine model (18); they conducted an mRNA microarray analysis and validated the findings by quantitative real-time PCR. A/J mice were exposed to either sidestream tobacco smoke or to filtered ambient air in whole-body chambers for 3 to 20 weeks, and mRNA was extracted from whole lungs following each exposure period. Tobacco exposure for these short time periods does not

generally result in lung cancer in A/J mice but may cause many preneoplastic changes.

Although the 15,000-gene array used in this study did not contain every gene of potential interest in lung carcinogenesis [e.g., *Cyp1a1*, a major contributor to benzo(a)pyrene and estrogen metabolism, and *Cyp19a1*, the aromatase gene], a number of potentially interesting genes were found to be altered by tobacco exposure. *Cyp1b1*, which encodes an enzyme that not only metabolizes tobacco smoke constituents but also is the primary enzyme responsible for generating catechol estrogens, was the major gene induced at each time point following tobacco exposure. At 3, 8, and 20 weeks of tobacco smoke exposure, *Cyp1b1* mRNA was consistently increased ~20-fold over mRNA extracted from lungs exposed to ambient air. *Cyp1b1* is also induced by tobacco exposure in humans. Although it is not surprising that a gene involved in the metabolism of tobacco constituents was induced, the implications of high induction of *Cyp1b1* are important because they relate to estrogen action in the lungs. The relative biological importance of this observation is underscored by comparison with the substantially lesser extent to which other genes were induced. Of all genes analyzed in the lungs, those showing the next highest degree of induction were *Cry1* and *Cbr3*, which showed mRNA levels ~2-fold higher than control levels at all three time points. *Cry1* is a circadian rhythm gene that affects cell cycle control, and *Cbr3* encodes a protein involved in the metabolism of xenobiotics and endogenous compounds. Melatonin, a regulator of circadian rhythm, is also metabolized by CYP1B1, leading Meireles et al. to suggest that dysregulation of circadian rhythms might occur through upregulation of *Cyp1b1* mRNA by tobacco exposure.

The high level of induction of *Cyp1b1* mRNA in the lungs of mice exposed to tobacco smoke at time points before lung tumor development suggests a role for estrogen metabolites in early lung changes that lead to lung cancer. The clinical relevance of the *Cyp1b1* findings in mice is supported by similar findings in the oral cavity and lungs of smokers (refs. 19, 20; see Table 4 in ref. 20). Meireles et al. further investigated the presence of estrogens and ERs in murine airways. Using immunocytochemistry analysis, they found that β -estradiol was localized to airway epithelium, along with cytoplasmic ER- α and mainly nuclear ER- β expression. They used gas chromatography coupled with mass spectroscopy in detecting both β -estradiol and estrone in lung tissues. Although Meireles et al. did not report any data on differences in these estrogen pathway constituents after tobacco exposure, their data strongly suggest that the substrates for CYP1B1 action are present locally in airway cells at risk for lung cancer. Through both metabolism to active mutagens and activation of their signaling receptors, estrogens may act on their own or with tobacco carcinogens to promote lung cancer and/or cause its progression.

The important findings of Meireles et al. add to the body of literature suggesting that estrogens play a role

¹ Unpublished data.

in lung cancer. They also show how murine models could help address many unanswered research questions. (a) Are catechol estrogens with carcinogenic potential produced locally in airway tissues as a result of tobacco exposure, and can estrogen-related DNA adducts be detected? (b) Are there sex differences in lung induction of *Cyp1b1* or in levels of estrogen or its metabolites in the lung after tobacco exposure? A recent study of tobacco effects on the oral transcriptome found no significant sex difference in *Cyp1b1* induction, suggesting that sex differences in the production of carcinogenic estrogen metabolites are related to differences in levels of the pro-carcinogenic estrogen substrate rather than to differential *Cyp1b1* induction (20). (c) Do preinvasive lesions in the airways show evidence of induction of estrogens or genes in estrogenic pathways? (d) Is aromatase activity in the airways modulated by tobacco exposure? (e) Can catechol estrogens, alone or in combination with lung carcinogens, induce lung tumors in susceptible strains of mice? (f) Can the action of progesterone oppose that of estrogens in the lung?

Additional important research questions related to hormones and lung cancer also should be addressed: (a) How do circulating and intratumoral estrogen levels relate to lung cancer progression and survival? (b) What is the biological function of airway cytoplasmic ER- α ? (c) What role do androgens play in lung cancer, especially considering that androgens are substrates for aromatase (CYP19A1), which is found in the lung? (d) The aryl hydrocarbon receptor, which is induced by tobacco carcinogens, can complex with the ER only when it is bound to an estrogen, causing the estrogen-bound ER to undergo proteasomal degradation (21); therefore, could agents that decrease estrogen levels such as aromatase inhibitors (AI) have unintended consequences in the lungs of active smokers? In active smokers, if the unliganded ER is stabilized by the

AI-associated removal of estrogens, ER signaling could still occur as a result of kinase-mediated phosphorylation, a known pathway for estrogen-independent ER activation (22). In such a scenario, active smoking might oppose or even reverse the ability of AIs to prevent or treat lung cancer. In animal models and humans, it will be important to consider the presence of tobacco when studying estrogen modulation in the lung.

The work of Meireles et al. sheds light on mechanisms whereby steroid hormone pathways may participate in the multiple events contributing to the development of lung cancer. The possible cancer-promoting effects of estrogen and possible cancer-inhibiting effects of progesterone in the lungs of both women and men at different times during lung carcinogenesis are very likely complex, are most likely modulated by smoking, and extend beyond the well-described reproductive roles of these hormones. A greater understanding of these pathways will lead to the optimal use of hormonal manipulation for lung cancer treatment and prevention. Several clinical trials of antiestrogens for activity against lung cancer are ongoing, but much work is still needed to clarify the biology underlying steroid hormone actions in the lung.

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