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

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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 quinine 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.

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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

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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-1 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 mors compared with matching normal lung tissue from downregulation of the progesterone receptor in lung tumor development (4) but might become less effective as progesterone in HRT also might protect against lung can-
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about lung cancer risk are not clear, HRT formulations
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 pres-
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 quinine that forms DNA adducts and is a more po-
tent and longer-lasting activator of estrogen receptors
(ER; ref. 17).

In this issue of the journal, Meireles et al. examined early
differences 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 peri-
od. 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 carcino-
genesis [e.g., Cyp1a1, a major contributor to benzo(a)
pyrene and estrogen metabolism, and Cyp19a1, the ar-
omatase gene], a number of potentially interesting genes
were found to be altered by tobacco exposure. Cyp1b1,
which encodes an enzyme that not only metabolizes to-
brobacco 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 ex-
tent to which other genes were induced. Of all genes
analyzed in the lungs, those showing the next highest de-
gree of induction were Cry1 and Cbr3, which showed
mRNA levels ~2-fold higher than control levels at all
time points. Cry1 is a circadian rhythm gene that
affects cell cycle control, and Cbr3 encodes a protein in-
volved in the metabolism of xenobiotics and endoge-
nous 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 be-
fore 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 epithel-
ium, 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 sig-
naling 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 procarcinogenic 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 estrogen 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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References

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