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Smoking Out Reproductive Hormone Actions in Lung Cancer

Jill M. Siegfried, Ph.D.

Department of Pharmacology, University of Minnesota 6-120 Jackson Hall 321 Church St. S.E.
Minneapolis, MN 55455 Telephone: 612-624-2584 jsiegfri@umn.edu

Abstract

Experimental and population-based evidence has been steadily accumulating that steroid hormones are fundamentally involved in the biology of the lung. Both estrogen and progesterone receptors are present in normal and malignant lung tissue, and the reproductive hormones that bind these receptors have a role in lung development, lung inflammation, and lung cancer. The estrogen receptor β (ER β) was discovered in the 1990's as a novel form of ER that is transcribed from a gene distinct from ER α , the receptor previously isolated from breast tissue. Interestingly, ER β is the predominate ER expressed in normal and malignant lung tissue, while inflammatory cells that infiltrate the lung are known to express both ER α and ER β . Although there is evidence from animal models for the preferential effects of ER β in the lungs of females, human lung tumors from males often contain comparable numbers of ER β -positive cells and male-derived lung cancer cell lines respond to estrogens. Lung tumors from both males and females also express CYP19 (aromatase), the rate-limiting enzyme in estrogen synthesis that converts testosterone to estrone and β -estradiol. Thus, testosterone acts as a precursor for local estrogen production within lung tumors, independent of reproductive organs. This review discusses the recent literature findings concerning the biology of the ERs, aromatase, and the progesterone receptor (PR) in lung cancer and highlights the ongoing clinical trials and future therapeutic implications of these findings.

ER Expression in Lung Cancer

Two different genes encode the ER proteins ER α and ER β , and these ER proteins are expressed with different tissue distributions (1). Both ER subtypes bind β -estradiol, the most active form of estrogen, with high affinity. Multiple isoforms of ER α and ER β have been reported, including three ER α isoforms (2) and five ER β isoforms (3,4). Two lines of evidence suggest that ER β is the major functional ER in the lung. First, a large difference in expression of ER β mRNA and ER α mRNA was observed in human lung tissue during fetal development (5) and in the adult mouse lung (6), with ER β being the predominant form. Second, a prominent phenotype of the female ER β knockout ($-/-$) mouse is a lung abnormality: at three months of age, the lungs of these mice contain a decreased number of alveoli and a reduction in expression of key regulators of surfactant homeostasis (6). By age five months, both female and male mice show alveolar collapse and alterations in extracellular matrix (7), suggesting that estrogen does have a role in lung homeostasis in males as well as females. Since ER α knockout mice do not show these changes, they are thought to be due to lack of ER β protein. A suggestion that ER β is important in lung tumor formation comes from carcinogenesis experiments using the ER β $-/-$ mouse. In this model, female but not male offspring were protected against development of lung tumors after *in utero* exposure to the polycyclic hydrocarbon dibenzochrysene (8). Whether this effect is restricted to protection during development or is a general protective effect of ER β loss on lung cancer formation is unknown. Estrogen was also shown to induce heightened lung tumor formation in female mice exposed to the tobacco carcinogen benzo[a]pyrene. A 50% increase in tumor multiplicity in the lungs and a 60% increase in lung tumor incidence were induced by β -estradiol (9).

ER α and ER β proteins can be distinguished by selective antibodies, and there is general agreement from a series of published studies that the full-length 59 kDa ER β protein (the ER β -1 isoform) is expressed in most human NSCLC cell lines, and is frequently detected in tissue specimens of human NSCLCs from men as well as women (10-14). ER β protein is found at higher levels in lung tumors compared to matching normal lung tissue from the same patient (14), suggesting up-regulation in cancer. ER β protein in lung tumors is detected in both nuclear and cytoplasmic compartments, and smaller variants of 53-56 kDa are often co-expressed (10). These smaller variants are the ER β isoforms 2-5 (15), which do not have functional activity for classical activation of EREs, although they can heterodimerize with ER β -1 and enhance its transcriptional activity (3). It also appears that full-length ER β -1 protein prefers to dimerize with the other lower molecular weight isoforms, so their presence could be a strong modulator of ER β action (3). The frequency of expression and function of the different ER β isoforms in lung cancer is not well understood, because a comprehensive comparison of the five known ER β isoforms has not been undertaken. Although ER β isoforms 2-5 lack the helix structures that allow for agonist conformation in ER dimers that are required for transcriptional activation, it is unknown whether these isoforms can function in non-genomic signaling pathways (described below) to activate kinases or interact with Akt or other signaling molecules, or if they can modulate non-genomic signaling as heterodimers with ER β -1. The properties of the ER β isoforms are shown in Table 1. ER β -3 is found only in testis tissues, whereas the other isoforms have been found in breast, prostate and lung tissues, among others.

Whether there is lung tumor expression of full-length ER α is controversial. ER α staining of lung tumor tissues and cell lines was found primarily in the cytoplasm and on the cell membrane, with rare expression in the nucleus (10-14), and both mRNA and protein analysis showed ER α messages to be comprised of alternatively spliced variants (10). These variant isoforms lack the amino-terminus because the proteins are differentially detected by antibodies that recognize the ER α amino- and carboxy-terminal (10). Immunoblotting of cell lines failed to detect the expected 66kD ER α protein, while smaller variants of 42kD and 54kD were found (16,17). Pro-tumorigenic effects such as estrogen-mediated RNA transcription of proliferation genes, non-genomic signaling that activates tyrosine kinases, and cell division in lung tumor cell lines can all be blocked by the ER inhibitor fulvestrant, demonstrating that ERs found in lung cancer are functional (10,16,17). A study of ER α and ER β selective agonists show that biological effects are predominantly mediated by ER β (17). Although ER α protein may be detected in a subset of lung tumors in patients, such as those with EGFR mutation (18), ER β appears to be the major ER expressed in lung cancer and responsible for mediating pro-tumor effects of estrogen. This is in contrast to breast and colon tissues, where ER β is thought to oppose proliferative effects of ER α . This may be due to differential recruitment of co-activators to ER β in the lung (16, 17) compared to other tissues, to the absence of full-length functional ER α protein in most lung tumors, or to the predominance of nongenomic signaling to cytoplasmic growth factor pathways in lung cancer through ER β .

There are now many published reports examining ER status in relation to NSCLC patient survival. Nuclear ER β staining was scored in the majority of lung cancer cases (11-14,19) and found to be associated with favorable prognosis in some studies. The prognostic significance was often limited to a subset of patients with a particular mutation, or only observed in male patients (12-14). However, most studies utilized antibodies to total ER β that could not distinguish different ER β isoforms. Recently, high cytoplasmic ER β -1 staining was identified as an independent negative prognostic factor for lung cancer (19). The negative effect on survival of ER β -1 was observed in male and female patients and showed no interaction with sex. Prognostic significance of cytoplasmic ER protein may be related to the importance of non-genomic signaling for ER action in the lung. Isoform

specificity was also reported in a study demonstrating that ER β -1, but not ER β -2, was related to worse prognosis in female stage I lung cancer patients (20). Nuclear ER β -1 also correlated with poor survival in metastatic lung cancer, but not early stage lung cancer patients (21). In contrast, the ER β -2 and -5 isoforms have been linked to better lung cancer outcome (15), although the mechanistic basis for this effect is unknown.

There is no consensus on survival effects of differences in expression of ER α protein in lung tumors, which as noted above is predominantly found as smaller variant proteins. It is variously reported that ER α has no effect on survival, or to correlate with poor prognosis (13,14,19). Nuclear and cytoplasmic ERs may have distinct functions and each component should be assessed both separately and together in lung cancer patient tissue specimens. Additionally the isoform type may be critical to interpreting survival data. A growing literature also shows that ERs localize to mitochondria and that estrogen can induce expression of the mitochondrial genome as well as increase vulnerability to oxidative stressors such as hydrogen peroxide. Recently, reports of mitochondrial action of ER β in lung cancer cells have suggested it appears to protect against apoptosis (22) and to show reduced activity during allergic airway inflammation in a mouse model of asthma (23). Analysis of the different ER β isoforms as well as their cellular localization will be necessary to completely understand the role of ER β in lung cancer. If standardized approaches can be developed, these hormone receptor markers may become useful biomarkers, potentially able to predict the aggressiveness of lung cancers and to identify patients who might be candidates for hormonal therapy.

ER Signaling in Lung Cancer

ERs belong to the nuclear steroid receptor superfamily, and signal to produce cellular responses following binding of estrogenic hormones (24). ERs function through several different mechanisms. As transcription factors, ERs bind estrogen response elements (EREs) and recruit co-activators that facilitate gene transcription. The ability of ERs to bind EREs is dependent upon ER activation, which occurs following ligand binding. Evidence that the lung is an estrogen-responsive tissue was seen in the transgenic ERE-luciferase reporter mouse, which used luciferase expression as an indicator of ERE activation following estrogen treatment. A 15-fold induction of reporter gene expression following treatment with estrogen was observed in the lungs of both male (25) and female (26) mice. ERs can also be activated through kinase phosphorylation, a process that does not require estrogen binding, and the phosphorylated ER functions as an estrogen-independent transcription factor (1). Growth factors that activate tyrosine kinase receptors such as EGFR or IGFR-1 can initiate signaling to downstream kinase pathways, which subsequently phosphorylate the ER and allow it to signal independently of estrogen. For example, EGFR can directly phosphorylate ER at specific serine residues (27). These residues were found to be phosphorylated in 87.5% of ER positive lung tumors examined, suggesting that EGFR activation of ER is common in lung cancer (28).

The nuclear actions of ERs involve changes in gene transcription that take place over several hours or longer through direct binding of ERs to promoter elements of estrogen response genes. However, estrogen can also rapidly activate cytoplasmic kinase signaling in seconds to minutes. This rapid signaling, termed non-genomic, occurs via non-nuclear ERs located in the membrane or the cytoplasm. In breast cancer cells, an additional membrane ER was identified as a G protein-coupled receptor named GPR30 (29). Expression of GPR30 has recently been demonstrated in lung cancer cells but the function and regulation of GPR30 in the lung is still unknown (30), and some data suggest that GPR30 exists in a complex with an ER and is not an independent receptor (31). In NSCLC cells, extra-nuclear ERs have been identified in plasma membrane fractions and cytoplasmic fractions. Treatment with

estrogen or ER β -specific ligands has been shown to promote stimulation of tyrosine kinase signaling pathways within 5-10 minutes (17, 32). These effects can be inhibited by the addition of fulvestrant, an ER antagonist.

Non-genomic ER signaling occurs via activation of tyrosine kinase growth factor pathways, such as the epidermal growth factor receptor (EGFR/HER-1) and the insulin-like growth factor 1 (IGF-1R). EGFR is a member of the tyrosine kinase receptor family that also includes HER-2, HER-3 and HER-4 (33), and many lung tumors are highly dependent on these pathways for growth and survival (34). In lung cancer cells (28,32,35), estrogen can rapidly activate the EGFR through release of EGFR ligands, and the combination of the anti-estrogen fulvestrant and an EGFR tyrosine kinase inhibitor such as gefitinib or erlotinib can maximally inhibit cell proliferation, induce apoptosis and reduce downstream signaling pathways both *in vitro* and *in vivo* (35,36). Erlotinib, the EGFR inhibitor that is FDA-approved for NSCLC, gave the best anti-tumor effects in NSCLC tumor xenografts when combined with fulvestrant (35,36). The multi-targeted tyrosine kinase inhibitor, vandetanib, which targets EGFR and VEGFR, also showed additive effects when combined with fulvestrant (37). A synergistic effect of gefitinib combined with the reversible non-steroidal aromatase inhibitor, anastrozole, was also observed in lung cancer cell lines, further suggesting a functional interaction between EGFR and ER pathways (38). Additionally, membrane ERs were co-localized with EGFR in lung tumors (32).

Aromatase in Lung Cancer

CYP19 (aromatase), a member of the cytochrome P450 family, catalyzes the conversion of androstenedione and testosterone to estrone and β -estradiol, respectively. Both CYP19 mRNA and protein have been detected in the lung (39,40). High β -estradiol levels were detected by mass spectroscopy in intra-tumoral extracts of primary NSCLC (41), and β -estradiol and its metabolites have also been detected in cell lines derived from lung tumors (42). Aromatase protein was detected in both NSCLC cell lines and tumor tissues, and the aromatase positive NSCLC cell lines were shown to secrete β -estradiol (43). The aromatase inhibitor anastrozole caused a substantial inhibition in growth of aromatase positive NSCLC tumor xenografts (43). Anastrozole was also demonstrated to prevent lung carcinogenesis in female mice exposed to a tobacco carcinogen; this effect was further increased by the addition of the ER antagonist fulvestrant (44). Interestingly, in this animal model of lung cancer prevention, aromatase expression was localized almost exclusively to macrophages and plasma cells that infiltrated preneoplastic and neoplastic areas of the lungs. Some aromatase protein localized to the abnormal epithelium, but at reduced intensity compared to the inflammatory cells. (44). An important source of estrogen synthesis may therefore be pulmonary inflammatory cells that infiltrate the lungs in response to carcinogens. This local production of estrogen may be part of the chronic inflammatory reaction occurring throughout the process of lung cancer development. Coombes and colleagues reported a lower incidence of primary lung cancer in breast cancer patients who were treated with the aromatase inhibitor exemestane following several years of tamoxifen therapy compared with those women who continued tamoxifen treatment (45). This suggests that aromatase inhibitors could suppress the development of lung cancer in post-menopausal women.

Mah et al. (46) found aromatase to be a predictive biomarker of lung cancer survival in older women with early stage lung cancer. Women over age 65 with lower levels of aromatase in tumor tissue had a greater chance of survival compared to those with higher aromatase expression. The prognostic value of aromatase expression was greatest in Stage I and II lung cancer patients. In this post-menopausal population of patients whose circulating estrogen levels are low due to decreased production by the ovaries, local estrogen production through tumor expression of aromatase could be an important determinant of estrogen levels. In a

separate study that included all stages of lung cancer, no general association between aromatase and lung cancer survival was observed unless combined with other markers such as ER β , EGFR and PR expression (19). No effect of sex or menopausal status was found for aromatase in this study. However, the study did not focus on older post-menopausal women. On the whole, the literature about aromatase in NSCLC strongly suggests that in the right population, aromatase inhibitors approved for therapeutic use in breast cancer, would also be useful to treat or possibly prevent lung cancer. Since aromatase inhibitors are contraindicated in premenopausal women and in men, this approach would be targeted to post-menopausal women whose lung tumors are aromatase positive, and may give clinicians a biomarker to predict survival of post-menopausal women at an early stage of disease where more treatment options are available. A phase I clinical trial is underway that will evaluate side effects and best dose of the irreversible steroidal aromatase inhibitor exemestane in combination with chemotherapy for late stage lung cancer therapy (NCT01664754). Other enzymes involved in metabolism and synthesis of estrogens are also under investigation in relation to lung cancer, such as 17 β -hydroxysteroid dehydrogenase type 1 (47).

PR in Lung Cancer

The two major isoforms of PR, PR-A and PR-B, play different roles in modulating cellular responses to progesterone. PR is an estrogen response gene, and PR-positive breast cancers are usually more differentiated tumors that respond to anti-estrogen therapy. The ratio of PR-A:PR-B is thought to affect clinical outcome for breast cancer, with high levels of PR-A associating with more differentiation and better survival. There are several reports of expression of total PR by primary NSCLC tissues, although there is a great deal of variability in the reported frequency of expression (19,48-51). Several reports found little or no PR in NSCLC (14,52,53), while lower PR was observed in lung tumors compared to matched normal lung tissue (19). Two reports have shown that PR is a strong protective factor for lung cancer (19,52). The antibodies used in these lung cancer survival studies do not distinguish between the PR-A and PR-B isoforms, which could exert different functions. Enzymes capable of synthesizing progesterone were also detected in many NSCLC tumors, and a positive correlation was observed between intratumoral levels of progesterone and the presence of three enzymes that participate in progesterone synthesis: steroidogenic acute regulatory protein, P450 side chain cleavage and 3 β -hydroxysteroid dehydrogenase (52). Progesterone treatment led to growth inhibition of tumor xenografts and along with induction of apoptosis, in agreement with clinical data suggesting presence of PR was correlated with longer overall survival in NSCLC patients (52). Progesterone has also been shown to inhibit migration and invasion of lung cancer cell lines (54). In breast cancer, PR is known to signal through ligand-independent mechanisms due to phosphorylation by kinases (55). One mechanism for low tumor PR expression in breast tumors is through increased growth factor signaling which leads to a more aggressive tumor biology with faster progression (56). Whether or not this same mechanism occurs in lung tumors is unknown and is currently being investigated.

Progesterone derivatives have been useful in the treatment of both endometrial cancer and breast cancer (57,58). Agents such as medroxyprogesterone acetate, which can be given orally, have potential for treatment of lung cancer, perhaps in combination with agents that suppress either the ER pathway or act on growth factor pathways such as EGFR, c-Met, or other TKIs. Whether progesterone could be used for prevention is open to debate since it can also have angiogenic properties.

Reproductive Hormones and Lung Cancer Survival

It is well established that women with advanced NSCLC live longer than men (59), although this observation is not specific to lung cancer, but is found in many tumor types. How much of this survival difference might be attributed to hormonal differences is not clear, and whether this survival difference is caused by differences between men and women in how cancer progresses, or to processes that generally lead to longer lifetimes for women compared to men is also unknown. A study seeking to address hormonal aspects of survival examined lung cancer presentation in pre-versus post-menopausal women. The results showed more advanced disease including poorly differentiated tumors with less favorable histologies occurred more often in pre-menopausal than post-menopausal women (60). Despite this, a significant survival difference between pre- and post-menopausal women was not seen. In a related study, women over the age of 60 had a significant survival advantage over both men and younger women, a difference potentially attributable to hormonal status since men did not show survival differences by age (61).

Several studies have now reported that exposure to hormone replacement therapy (HRT) was associated with negative effects on lung cancer survival. Ganti et al. (62) reported that a significant association between both a lower age at lung cancer diagnosis and a shorter survival time in women who used HRT around the time of diagnosis versus those who did not. This effect was more apparent in women who smoked, suggesting an interaction between estrogens and tobacco carcinogens. In a randomized, placebo-controlled trial in which more than 16,000 post-menopausal women received placebo or daily HRT for 5 years (the Women's Health Initiative), a strong negative effect on survival after a lung cancer diagnosis was observed in women on the HRT arm (63). The HRT group had a significantly greater likelihood of dying from lung cancer compared to placebo, group with a trend toward more lung cancer diagnoses in the HRT arm. A role for estrogen in lung cancer presentation is supported by several retrospective population studies demonstrating that anti-estrogen use improves survival of female lung cancer patients. An observational study, which included more than 6,500 breast cancer survivors, found that women who received any anti-estrogen treatment had significantly lower subsequent lung cancer mortality (64). The Manitoba Cancer Registry also evaluated 2,320 women with or without exposure to anti-estrogens (65). Anti-estrogen use both before and after lung cancer diagnosis was significantly associated with decreased lung cancer mortality. Published studies on both HRT and anti-estrogen use support the idea of estrogen acting as a promoter of lung cancer aggressiveness that may play a key role not only in the biology but also the outcome of lung cancer.

Effects of hormone replacement that promote poor outcomes in lung cancer could be due to both direct effects on tumor proliferation as well as indirect effects caused by response of the microenvironment to estrogen. For example, endothelial cells show greater survival in the presence of β -estradiol (66), an effect that could increase angiogenesis. ER β was critical for progression of lung cancer metastasis in the brain, due in large part to a contribution from astrocytes that express ER β (67). Estrogen and progesterone mediated resistance of lung cancer cells to apoptosis induced by cisplatin, a major chemotherapy drug used to treat lung cancer (68).

Reproductive Hormones and Lung Cancer Risk

While many studies suggest that exposure to exogenous hormones is detrimental to *survival* of lung cancer patients, whether exogenous hormone use prior to diagnosis affects *the risk* of developing lung cancer is controversial. In the Women's Health Initiative study described earlier, the HRT arm had an increase in incidence of lung cancer compared to placebo, but it

was not statistically significant (63). Two other large cohorts with HRT exposure data have also been examined for relationship to risk of a later lung cancer. The Vitamins and Lifestyle Study followed 36,000 post-menopausal women who filled out baseline questionnaires about hormone use, and were assessed for lung cancers diagnosed up to 7 years after baseline data were collected. The California Teachers Study consisted of over 60,000 post-menopausal women who supplied baseline data about hormone use and were assessed for lung cancer diagnoses up to 11 years after baseline. In contrast to the Women's Health Initiative, a significant increase in lung cancer incidence associated with HRT was observed in the Vitamins and Lifestyle Study, and this effect on lung cancer risk was duration dependent (69), while in the California teachers cohort, no effect of HRT on lung cancer risk was observed (70). Two other case-control reports suggest that HRT use prior to diagnosis could protect women from developing lung cancer (71,72). In one study, a protective effect was especially found in women who smoked (71), and an inverse relationship was also observed between HRT use and NSCLC risk in postmenopausal women with ER-positive, but not ER-negative lung tumors (72). A recent pooled analysis of six case-control studies (1961 cases and 2609 controls) from the International Lung Cancer Consortium found a strongly reduced lung cancer risk associated with both oral contraceptive use and HRT use, independent of smoking status or body mass index. (73).

It is interesting that case-control studies show protective effects of exogenous hormone use, while cohort studies do not, or show detrimental effects. In the pooled case-control analysis (73), neither duration of use nor dose were factors in the degree of protection, suggesting short exposures were equally protective, while in cohort studies a longer duration was more detrimental for either lung cancer incidence or survival. There could be differences in the balance between pro-cancer and anti-cancer effects of estrogen in normal lung epithelium compared to malignant epithelium, that could change over time. ER β is over-expressed in lung tumors compared to matched normal lung tissues (19), which could lead to abnormal responses to estrogen in malignant cells that promote lung cancer. ER β expression is induced by oxidative stress and inflammation in both endothelial cells and macrophages, which could also lead to pro-cancer responses to estrogens in areas of the lung that are at-risk for lung cancer development. However, the immune system is also regulated by estrogen, and the ability of the immune system to reject malignant lung tissues early in the cancer process could be enhanced by hormone use. Depending upon context, estrogen can either suppress or enhance inflammation, and estrogen can also down-modulate the release of other growth factors that contribute to cancer development. Since lung tumors are known to produce aromatase, it is possible that exogenous hormone use reduces estrogen production in the airways by inhibiting pulmonary aromatase expression. This could also repress production of hydroxyl forms of estrogen, which are only produced in the periphery, and which also have pro- and anti-cancer effects (74). The balance of these processes may contribute to whether hormone use results in protection from or promotion of lung cancer. Since it is now recommended that HRT use be of limited duration in post-menopausal women, due to general health hazards of long-term use, HRT effects on lung cancer risk or outcome may be less pronounced in the future.

Clinical Trials Using the ER Antagonist Fulvestrant in Lung Cancer

The body of evidence showing proliferative effects of estrogen on lung tumors and an interaction between ER and EGFR signaling in lung cancer suggests that targeting both pathways could be beneficial for therapy. Targeting the EGFR using TKIs as a single therapy is of limited use in the absence of an EGFR mutation, which occurs in about 20% of adenocarcinoma patients. The patients who respond well to EGFR TKIs are mainly females and never smokers (76), which may relate to cross-talk in signaling between the EGFR and ER in lung cancer (75). As noted above, some studies have reported a correlation between

EGFR mutation and ER expression (18,76). Knowledge of ER-EGFR cross-talk was translated in a phase I clinical trial using drugs that target EGFR and ER, that assess the toxicity of combined treatment of gefitinib with fulvestrant (77). Targeting both pathways was found to be safe and to have anti-tumor activity in female patients with advanced, pre-treated NSCLC. Additionally, high ER β expression was correlated with better patient survival in this cohort of fulvestrant-treated patients. A phase II trial examining the combination of erlotinib (the now-preferred EGFR TKI) with fulvestrant compared to erlotinib alone has recently been completed, in which 100 patients were treated (78). Combination treatment was well tolerated. Progression-free survival and response rate were similar between the two treatment arms in unselected patients. However, among patients with EGFR wild-type tumors, the clinical benefit rate (which included partial responders and those with stable disease) was significantly higher among patients treated with the combination compared to erlotinib alone, with trends towards improved survival. It is yet to be determined what ER-related biomarkers will be informative in defining the patients most likely to benefit. These clinical trials suggest that targeting the ER pathway in conjunction with the EGFR pathway, or other aberrantly expressed tyrosine kinase receptors, will have beneficial antitumor effects in NSCLC as has been observed in breast cancer cells (79), particularly in patients whose tumors do not contain an EGFR mutation. The combination of anti-estrogen therapy or aromatase inhibitors with targeted therapies that block different growth factor receptors also warrants clinical investigation.

Summary

A body of preclinical evidence now demonstrates that estrogen is a driver of lung cancer. Estrogens induce cell proliferation of NSCLC cells in cell culture (10,32), human tumor xenografts (10), and in animal models of lung cancer (9, 80). Estrogen can also modulate expression of genes in NSCLC cell lines that are important for inducing cell proliferation such as c-myc and cyclin D1 (16). Estrogen signaling through ERE and AP-1 promoter elements was shown to occur primarily through ER β in NSCLC cells, as was enhanced lung tumor xenograft growth (17,35). In addition, fulvestrant, a pure ER antagonist, inhibits proliferation in NSCLC cell lines and in lung tumor xenograft models in immunocompromised mice (10). This preclinical evidence combined with population data showing that HRT use reduces lung cancer survival strongly support targeting the ER pathway therapeutically. Several ongoing trials seek to find biomarkers that will identify the lung cancer patients most likely to benefit from abrogation of hormone pathways.

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References

1. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett.* 1996; 392:49–53. [PubMed: 8769313]
2. Lin AHY, Li RWS, Ho EYW, Leung GPH, Leung SWS, Vanhoutte PM, Man RYK. Differential ligand binding affinities of human estrogen receptor- α isoforms. *PLOS One.* 2013; 8:e63199. [PubMed: 23646196]
3. Leung YK, Mak P, Hassan S, Ho SM. Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling. *Proc Natl Acad Sci USA.* 2006; 103:13162–7. [PubMed: 16938840]
4. Shaaban AM, Green AR, Karthik S, Alizadeh Y, Hughes TA, Harkins L, Ellis IO, Robertson JF, Parish EC, Saunders PT, Groome NP, Speirs V. Nuclear and cytoplasmic expression of ERbeta1, ERbeta2, and ERbeta5 identifies distinct prognostic outcome for breast cancer patients. *Clin Cancer Res.* 2008; 14:5228–35. [PubMed: 18698041]

5. Brandenberger AW, Tee MK, Lee JY, Chao V, Jaffe RB. Tissue distribution of estrogen receptors alpha (ER- α) and beta (ER- β) mRNA in the midgestational human fetus. *J Clin Endocrin Metab.* 1997; 82:3509–12.
6. Patrone C, Caael TN, Pettersson K, Piao YS, Cheng G, Ciana P, Maggi A, Warner M, Gustafsson JA, Nord M. Regulation of postnatal lung development and homeostasis by estrogen receptor β . *Mol Cell Biol.* 2003; 23:8542–52. [PubMed: 14612399]
7. Morani A, Barros RP, Imamov O, et al. Lung dysfunction causes systemic hypoxia in estrogen receptor beta knockout (ERbeta $^{-/-}$) mice. *Proc. Natl Acad Sci USA.* 2006; 103:7165–9. [PubMed: 16636272]
8. Benninghoff AD, Williams DE. The role of estrogen receptor β in transplacental cancer prevention by indole-3-carbinol. *Cancer Prev Res.* 2013; 6:339–48.
9. Chen Z, Zhang Y, Yang H, et al. Estrogen promoted benzo[a]pyrene-induced lung carcinogenesis through oxidative stress damage and cytochrome c-mediated caspase-3 activation pathways in female mice. *Cancer Letters.* 2011; 308:14–22. [PubMed: 21601985]
10. Stabile LP, Davis AL, Gubish CT, Hopkins TM, Luketich JD, Christie N, Finkelstein S, Siegfried JM. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor alpha and beta and show biological responses to estrogen. *Cancer Res.* 2002; 62:2141–50. [PubMed: 11929836]
11. Wu CT, Chang YL, Shih JY, Lee YC. The significance of estrogen receptor beta in 301 surgically treated non-small cell lung cancers. *J Thorac Cardiovasc Surg.* 2005; 130:979–86. [PubMed: 16214508]
12. Schwartz, AG.; Prysak, GM.; Murphy, V.; Lonardo, F.; Pass, H.; Schwartz, J.; Brooks, S. Nuclear estrogen receptor beta in lung cancer: expression and survival differences by sex.
13. Kawai H, Ishii A, Washiya K, Konno T, Kon H, Yamaya C, Ono I, Ogawa J. Combined overexpression of EGFR and estrogen receptor alpha correlates with a poor outcome in lung cancer. *Anticancer Res.* 2005; 25:4693–98. [PubMed: 16334162]
14. Skov BG, Fischer BM, Pappot H. Oestrogen receptor β over expression in males with non-small cell lung cancer is associated with better survival. *Lung Cancer.* 2008; 59:88–94. [PubMed: 17905466]
15. Liu Z, Liao Y, Tang H, et al. The expression of estrogen receptors β 2,5 identifies and is associated with prognosis in non-small cell lung cancer. *Endocrine.* 2013 [Epub ahead of print].
16. Hershberger PA, Vasquez AC, Kanterewicz B, Land S, Siegfried JM, Nichols M. Regulation of endogenous gene expression in human non-small cell lung cancer cells by estrogen receptor ligands. *Cancer Res.* 2005; 65:1598–605. [PubMed: 15735050]
17. Hershberger PA, Stabile LP, Kanterewicz B, et al. Estrogen receptor beta (ERbeta) subtype-specific ligands increase transcription, p44/p42 mitogen activated protein kinase (MAPK) activation and growth in human non-small cell lung cancer cells. *J Steroid Biochem Mol Biol.* 2009; 116:102–9. [PubMed: 19460433]
18. Mazieres J, Rouquette I, Lepage B, et al. Specificities of lung adenocarcinoma in women who have never smoked. *J Thorac Oncol.* 2013; 8:923–9. [PubMed: 23608817]
19. Stabile LP, Dacic S, Land SR, et al. Combined analysis of estrogen receptor β -1 and progesterone receptor expression identifies lung cancer patients with poor outcome. *Clin Cancer Res.* 2011; 17:154–64. [PubMed: 21062926]
20. Sethi S, Coti M, Lonardo F. Expression of estrogen receptor beta 1 but not estrogen receptor beta 2 or alpha is linked to worse prognosis in stage I adenocarcinoma, in women, in a large epidemiological cohort but not in a smaller, single hospital based series. United States and Canadian Academy of Pathology. 2010 Abstract 1843.
21. Navaratnam S, Skliris G, Qing G, et al. Differential role of estrogen receptor beta in early versus metastatic non-small cell lung cancer. *Horm Cancer.* 2012; 3:93–100. [PubMed: 22302352]
22. Zhang G, Yanamala N, Lathrop KL, et al. Ligand-independent antiapoptotic function of estrogen receptor-beta in lung cancer cells. *Mol Endocrinol.* 2010; 24:1737–47. [PubMed: 20660297]
23. Simoes DC, Psarra AM, Mauad T, et al. Glucocorticoid and estrogen receptors are reduced in mitochondria of lung epithelial cells in asthma. *PLoS One.* 2012; 7:e39183. [PubMed: 22761735]

24. Blaustein JD. Steroid hormone receptors: long- and short-term integrators of the internal milieu and the external environment. *Horm. Metab. Res.* 2012; 44:563–8. [PubMed: 22549398]
25. Lemmen JG, Arends RJ, van Boxtel AL, van der Saag PT, van der Burg B. Tissue- and time-dependent estrogen receptor activation in estrogen reporter mice. *J Mol Endocrinol.* 2004; 32:689–701. [PubMed: 15171709]
26. Ciana P, DiLuccio G, Belcredito S, Pollio G, et al. Engineering of a mouse for the in vivo profiling of estrogen receptor activity. *Molec Endocrinol.* 2001; 15:1104–13. [PubMed: 11435611]
27. Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H, Metzger D, Chambon P. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science.* 1995; 270:1491–4. [PubMed: 7491495]
28. Marquez-Garban DC, Chen H-W, Fishbein MC, Goodglick L, Pietras RJ. Estrogen receptor signaling pathways in human non-small cell lung cancer. *Steroids.* 2007; 72:135–43. [PubMed: 17276470]
29. Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology.* 2005; 146:624–32. [PubMed: 15539556]
30. Jala VR, Radde BBN, Haribabu B, et al. Enhanced expression of G-protein coupled estrogen receptor (GPER/GPR30) in lung cancer. *BMC Cancer.* 2012; 12:624. 2012. [PubMed: 23273253]
31. Kang L, Zhang X, Xie Y, et al. Involvement of estrogen receptor variant ER- α 36, Not GPR30, in nongenomic estrogen signaling. *Molec Endocrinol.* 2010; 24:709–21. [PubMed: 20197310]
32. Pietras RJ, Marquez-Garban DC. Membrane-associated estrogen receptor signaling pathways in human cancers. *Clin Cancer Res.* 2007; 2007; 13:4672–76. [PubMed: 17699844]
33. Rajkumar T, Gullick WJ. The type I growth factor receptors in human breast cancer. *Breast Cancer Res Treat.* 1994; 29:3–9. [PubMed: 7912566]
34. Rosell R, Bivona TG, Karachaliou N. Genetics and biomarkers in personalisation of lung cancer treatment. *Lancet.* 2013; 382:720–31. [PubMed: 23972815]
35. Stabile LP, Lyker JS, Gubish CT, Zhang W, Grandis JR, Siegfried JM. Combined targeting of the estrogen receptor and the epidermal growth factor receptor in non-small cell lung cancer shows enhanced antiproliferative effects. *Cancer Res.* 2005; 65:1459–70. [PubMed: 15735034]
36. Garon EB, Pietras RJ, Finn RS, et al. Antiestrogen fulvestrant enhances the antiproliferative effects of epidermal growth factor receptor inhibitors in human non-small cell lung cancer. *J Thorac Oncol.* 2013; 8:270–8. [PubMed: 23399957]
37. Siegfried JM, Gubish CT, Rothstein ME, et al. Combining the multitargeted kinase inhibitor vandetanib with the antiestrogen fulvestrant enhances its antitumor effect in non-small cell lung cancer. *J Thorac Oncol.* 2012; 7:485–95. [PubMed: 22258476]
38. Shen L, Li Z, Shen S, et al. The synergistic effect of EGFR tyrosine kinase inhibitor gefitinib in combination with aromatase inhibitor anastrozole in non-small cell lung cancer cell lines. *Lung Cancer.* 2012; 78:193–200. [PubMed: 22985911]
39. Martel C, Melner MH, Gagné D, Simard J, Labrie F. Widespread tissue distribution of steroid sulfatase, 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase (3 beta-HSD), 17 beta-HSD 5 alpha-reductase and aromatase activities in the rhesus monkey. *Mol Cell Endocrinol.* 1994; 104:103–11. [PubMed: 7821701]
40. Price T, Aitken J, Simpson ER. Relative expression of aromatase cytochrome P450 in human fetal tissues as determined by competitive polymerase chain reaction amplification. *J Clin Endocrinol Metab.* 1992; 74:879–83. [PubMed: 1548354]
41. Niikawa H, Suzuki T, Miki Y, et al. Intratumoral estrogens and estrogen receptors in human non-small cell lung carcinoma. *Clin Cancer Res.* 2008; 14:4417–26. [PubMed: 18579664]
42. Xu X, Veenstra T.D. Concentration of endogenous estrogens and estrogen metabolites in the NCI-60 human tumor cell lines. *Gen Med.* 2012; 4:31.
43. Weinberg OK, Marquez-Garban DC, Fishbein MC, Goodglick L, Garban HJ, Dubinett SM, Pietras RJ. Aromatase inhibitors in human lung cancer therapy. *Cancer Res.* 2005; 65:11287–91. [PubMed: 16357134]

44. Stabile LP, Rothstein ME, Cunningham DE, et al. Prevention of tobacco carcinogen-induced lung cancer in female mice using antiestrogens. *Carcinogenesis*. 2012; 33:2181–9. [PubMed: 22859269]
45. Coombes RC, Hall E, Gibson LJ, et al. A randomized trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. *N Engl J Med*. 2004; 350:11:1081–92. [PubMed: 15014181]
46. Mah V, Seligson DB, Li A, Marquez DC, Wistuba II, Eishimali Y, Fishbein MC, Chia D, Pietras RJ, Goodglick L. Cancer Research. Aromatase expression predicts survival in women with early-stage non-small cell lung cancer. 2007; 67:10484–90.
47. Verma MK, Miki Y, Abe K, et al. Intratumoral localization and activity of 17 β -hydroxysteroid dehydrogenase type 1 in non-small cell lung cancer: a potent prognostic factor. *J Transl Med*. 2013; 11:167. [PubMed: 23837683]
48. Su JM, Hsu HK, Chang H, Lin SL, Chang HC, Huang MS, Tseng HH. Expression of estrogen and progesterone receptors in non-small-cell lung cancer: immunohistochemical study. *Anticancer Res*. 1996; 16:3803–6. [PubMed: 9042261]
49. Kaiser U, Hofmann J, Schilli M, Wegmann B, Klotz U, Wedel S, Virmani AK, Wollmer E, Branschke D, Gazdar AF, Havemann K. Steroid-hormone receptors in cell lines and tumor biopsies of human lung cancer. *Int J Cancer*. 1996; 67:357–64. [PubMed: 8707409]
50. Di Nunno L, Larsson LG, Rinehart JJ, Beissner RS. Estrogen and progesterone receptors in non-small cell lung cancer in 248 consecutive patients who underwent surgical resection. *Arch Pathol Lab Med*. 2000; 124:1467–70. [PubMed: 11035577]
51. Raso MG, Behrens C, Herynk MH, et al. Immunohistochemical expression of estrogen and progesterone receptors identifies a subset of NSCLCs and correlates with EGFR mutation. *Clin Can Res*. 2009; 15:5359–68.
52. Ishibashi H, Suzuki T, Suzuki S, et al. Progesterone receptor in non-small cell lung cancer-a potent prognostic factor and possible target for endocrine therapy. *Cancer Res*. 2005; 65:6450–8. [PubMed: 16024650]
53. Abe K, Miki Y, Ono K, et al. Highly concordant coexpression of aromatase and estrogen receptor beta in non-small cell lung cancer. *Hum Pathol*. 2010; 41:190–8. [PubMed: 19800101]
54. Xie M, You S, Chen Q, et al. Progesterone inhibits the migration and invasion of A549 lung cancer cells through membrane progesterone receptor a-mediated mechanisms. *Oncol Rep*. 2013; 29:1873–80. [PubMed: 23467643]
55. Lange CA, Shen T, Horwitz KB. Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. *Proc Natl Acad Sci USA*. 2000; 97:1032–7. [PubMed: 10655479]
56. Cui X, Lazard Z, Zhang P, et al. Progesterone crosstalks with insulin-like growth factor signaling in breast cancer cells via induction of insulin receptor substrate-2. *Oncogene*. 2003; 22:6937–41. [PubMed: 14534541]
57. Sutton G. Hormonal aspects of endometrial cancer. *Curr Opin Obstet Gynecol*. 1990; 2:69–73. [PubMed: 2102309]
58. Kelley RM, Baker WH. Progestational agents in the treatment of carcinoma of the endometrium. *N Engl J Med*. 1961; 264:216–22. [PubMed: 13752346]
59. Albain KS, Crowley JJ, LeBlanc M, Livingston RB. Survival determinants in extensive-stage non-small-cell lung cancer: the Southwest Oncology Group experience. *J Clin Oncol*. 1991; 9:1618–26. [PubMed: 1651993]
60. Moore KA, Mery CM, Jaklitsch MT, Estocin AP, Bueno R, Swanson SJ, Sugarbaker DJ, Lukanich JM. Menopausal effects on presentation, treatment, and survival of women with non-small cell lung cancer. *Ann Thorac Surg*. 2003; 76:1789–95. [PubMed: 14667585]
61. Wakelee HA, Dahlberg SE, Schiller JA, Langer CJ, Sandler AB, Brahmer JR, Belani CP, Johnson DH. Menopausal status of women may affect survival in advanced NSCLC: Analysis of recent Eastern Cooperative Oncology Group (ECOG) studies using age of 60 years or older as a surrogate marker: P1-052. *J Thoracic Oncology*. 2007b; 2:S570.

62. Ganti AK, Sahmoun AE, Panwalkar AW, Tendulkar KK, Potti A. Hormone replacement therapy is associated with decreased survival in women with lung cancer. *J. Clin Oncol.* 2006; 24:59–63. [PubMed: 16314616]
63. Chlebowski RT, Schwartz AG, Wakelee H, et al. Estrogen plus progestin and lung cancer in postmenopausal women. *Lancet.* 2009; 375:1243–51. [PubMed: 19767090]
64. Bouchardy C, Benhamou S, Schaffar R, et al. Lung cancer mortality risk among breast cancer patients treated with anti-estrogens. *Cancer.* 2011; 117:1288–95. [PubMed: 21264820]
65. Lother SA, Harding GA, Musto G, et al. Antiestrogen use and survival of women with non-small cell lung cancer in Manitoba, Canada. *Horm Cancer.* 2013 [Epub ahead of print].
66. Spyridopoulos I, Sullivan AB, Kearney M, et al. Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis. *Circulation.* 1997; 95:1505–14. [PubMed: 9118519]
67. Xu L, Gao G, Ren J, et al. Estrogen reception β of host promotes the progression of lung cancer brain metastasis of an orthotopic mouse model. *J. Cancer Ther.* 2012; 3:352–58.
68. Grott M, Karayaka S, Mayer F, et al. Progesterone and estrogen prevent cisplatin-induced apoptosis of lung cancer cells. *Anticancer Res.* 2013; 33:791–800. [PubMed: 23482746]
69. Slatore CG, Chien JW, Au DH, et al. Lung cancer and hormone replacement therapy: association in the vitamins and lifestyle study. *J Clin Oncol.* 2010; 28:1540–6. [PubMed: 20159813]
70. Clauge J, Reynolds P, Sullivan-Halley J, et al. Menopausal hormone therapy does not influence lung cancer risk: results from the California teachers study. *Cancer Epi Biomarkers Prev.* 2011; 20:560–64.
71. Schabath MB, Wu X, Vassilopoulou-Sellin R, Vaporciyan AA, Spitz MR. Hormone replacement therapy and lung cancer risk: a case-control analysis. *Clin Cancer Res.* 2004; 10:113–23. [PubMed: 14734459]
72. Schwartz AG, Wenzlaff AS, Prysak GM, Murphy V, Cote ML, Brooks SC, Skafar DF, Lonardo F. Reproductive factors, hormone use, estrogen receptor expression and risk of non-small cell lung cancer in women. *Journal of Clinical Oncology.* 2007; 25:5785–92. [PubMed: 18089876]
73. Pestori AC, Carugno M, Conconi D, et al. Hormone use and risk for lung cancer: a pooled analysis from the International Lung Cancer Consortium (ILCCO). *Br. J. Cancer.* 2013; 109:1954–64. [PubMed: 24002594]
74. Cutolo M, Capellino S, Straub RH. Oestrogens in rheumatic diseases: friend or foe? *Rheumatology.* 2008; 47:iii2–5. [PubMed: 18504280]
75. Yang SH, Mechanic LE, Yang P, et al. Mutations in the tyrosine kinase domain of the epidermal growth factor receptor in non-small cell lung cancer. *Clin Cancer Res.* 2005; 11:2106–10. [PubMed: 15788655]
76. Nose N, Sugio K, Oyama T, et al. Association between estrogen receptor-beta expression and epidermal growth factor receptor mutation in the postoperative prognosis of adenocarcinoma of the lung. *J Clin Oncol.* 2009; 27:411–7. [PubMed: 19064969]
77. Traynor AM, Schiller JH, Stabile LP, et al. Pilot study of gefitinib and fulvestrant in the treatment of post-menopausal women with advanced non-small cell lung cancer. *Lung Cancer.* 2009; 64:51–9. [PubMed: 18701186]
78. Garon EB, Siegfried JM, Dubinett SM, et al. Results of TORI-L-03, a randomized, multicenter phase II clinical trial of erlotinib (E) or E + fulvestrant (F) in previously treated advanced non-small cell lung cancer (NSCLC).. Proceedings of the 104th Annual Meeting of the American Association for Cancer Research; Washington DC. Philadelphia (PA): AACR. 2013 Apr 6-10; 2013. Abstract nr 4664
79. Okubo S, Kurebayashi J, Otsuki T, Yamamoto Y, Tanaka K, Sonoo H. Additive antitumour effect of the epidermal growth factor receptor tyrosine kinase inhibitor gefitinib (Iressa, ZD1839) and the antiestrogen fulvestrant (Faslodex, ICI 182,780) in breast cancer cells. *Br J Cancer.* 2004; 90:236–44. [PubMed: 14710235]
80. Hammoud Z, Tan B, Badve S, Bigsby RM. Estrogen promotes tumor progression in a genetically defined mouse model of lung adenocarcinoma. *Endocr Relat Cancer.* 2008; 15:475–83. [PubMed: 18509000]

Table 1Properties of Estrogen Receptor β Isoforms

	Size (KD)	β -estradiol Binding Affinity	ERE Activation	Heterodimer Formation with ER β -1
ER β -1	59	High	Yes	Yes
ER β -2	56	None	No	Yes
ER β -3	57	None	No	Yes
ER β -4	54	Moderate	No	Yes
ER β -5	53	Moderate	No	Yes