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Lung cancer in women: role of estrogens

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Abstract

The incidence of lung cancer in females is increasing, in contrast to that seen in males. In addition, the proportion of lung cancer cases in women attributable to smoking is approximately half of that seen in males. Female sex hormones, especially estrogen, may play a key role in this. Estrogen receptors ER α and ER β have been detected on lung cancer cells and there is new evidence suggesting that hormone-replacement therapy may increase both the incidence of, and mortality from, lung cancer in women. Laboratory evidence lends credence to the carcinogenic effects of estrogens in lung cancer. This article summarizes the current evidence on their role in lung cancer.

Keywords

estrogen; estrogen receptors; fulvestrant; hormone-replacement therapy; lung cancer; women

Lung cancer is one of the most lethal human malignancies, owing to both its high incidence and high case–fatality rate. In 2005 alone, lung cancer killed 1.2 million people worldwide, representing 17.1% of all cancer-related deaths [1]. Lung cancer is the second most common cancer in women after breast cancer and is also the second leading cause of cancer-related deaths in women worldwide [1]. In the USA alone, an estimated 103,350 women developed lung cancer in 2009 and the majority, about 70,490, died of this disease, making it the leading cause of cancer-related deaths in the USA [2].

There has been a tremendous increase in the incidence of lung cancer in females over the last decade, which is probably related to the rise in tobacco-smoking rates post-World War II. While smoking habits in females differ from those in males, other gender-specific factors probably also play a role. For example, in the year 2000, an estimated 85% of lung cancers in men and 47% of lung cancers in women were attributable to tobacco smoking. This suggests the impact of non-tobacco related factors in lung carcinogenesis in women.

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The estimated numbers of lung cancer cases worldwide has increased by 51% since 1985 (44% in men compared with 76% in women). In men, this increase appears to be solely due to population growth and aging, with a small (3.3%) decrease in the actual age-standardized incidence rate. By contrast, the age-standardized incidence has increased by 22% in women. In men, several developed nations are now past the peak of the lung cancer epidemic, with declining incidence rates (e.g., the USA [3] and the countries of Northern and Western Europe [4]). However, in women, the epidemic is still in its early stages and most Western countries currently show a rising trend in incidence and mortality. The incidence rates in men decreased by 2.3% per year in the early 1990s. By contrast, a sharp increase in the incidence of lung cancer, from six per 100,000 in 1960 to more than 40 per 100,000 in 1990, has occurred in women. However, more recent data suggest that the incidence of lung cancer among women may have reached a plateau and that the peak of the epidemic may have already occurred in countries such as the USA, Spain and the UK [3,5].

One of the main biologic differences between men and women is the presence of female sex hormones and an increasing amount of evidence suggests that the female sex hormones – estrogens and progesterone – may play an important role in lung carcinogenesis. This review will describe the current data on the role of female sex hormones in the pathogenesis of lung cancer.

Hormonal factors in lung cancer

The most obvious biological differences between men and women are hormonal. With the increasing rates of lung cancer in women and their increased susceptibility to the detrimental effects of tobacco smoke compared with men, the role of female steroid sex hormones has been hypothesized to be a factor in lung carcinogenesis. It is well established that estrogens may act as direct carcinogens by either causing or modulating the effects of risk factors including chromosomal alterations and genetic mutations in breast and other female cancers.

Effect of female sex hormones on the incidence of lung cancer

The role of hormone-replacement therapy (HRT) and oral contraceptives (OCs) in the development of lung cancer is controversial [6]. An increased risk of lung cancer in women who received HRT was noted in a large-scale epidemiologic cohort study; however, no adjustment was made for the amount of smoking [7]. In a case-control study, 180 women who had lung cancer were evaluated for their history of smoking and HRT. For women who had never smoked, HRT posed no additional risk [6]. There was, however, a statistically significant synergy between smoking and HRT; women who smoked but did not take HRT had an odds ratio (OR) of 13 for developing lung cancer compared with an OR of 32.4 for women who smoked and took HRT. In addition, the investigators reported an increased risk of adenocarcinoma associated with HRT (OR: 1.7). Adami *et al.* have also shown an increased risk (relative risk [RR]: 1.26) of lung cancer in women receiving HRT in their study [7]. It appears that the higher circulating levels of estrogen in women compared with men, coupled with their lower rate of DNA repair, make women particularly susceptible to the carcinogenic influence of tobacco smoke. There are also data suggesting that HRT may actually exert a protective effect [8-11]. The studies evaluating the role of HRT on the incidence of lung cancer are summarized in TABLE 1.

In an interesting case-control analysis, Koushik *et al.* compared the effects of menopause on lung cancer risk in 422 women with lung cancer and 577 controls [12]. They found that while most characteristics of menstruation and pregnancy were not associated with lung cancer risk, an increased risk was observed for women who had had a non-natural menopause, which predominantly included women who had had a bilateral ovariectomy, compared with women who had had a natural menopause. They also observed an inverse

association of lung cancer risk with the age of menopause, that is, women who were less than 45 years of age at the onset of menopause had a higher risk of developing lung cancer compared with older women.

Role of female sex hormones on outcomes from lung cancer

In addition to their effects on incidence, endocrine factors may also affect outcomes in women with established lung cancer. Moore *et al.* analyzed the Surveillance, Epidemiology, and End Results database to evaluate the influence of menopausal status on outcome in lung cancer among women. Utilizing an average menopausal age of 51 years, they classified 14,676 women entered in the database into premenopausal and postmenopausal groups [13]. They found that premenopausal women had more extensive disease at presentation and had an increased frequency of adenocarcinoma compared with postmenopausal women. While premenopausal women and younger men had similar mortality, on multivariate analysis, postmenopausal women had fewer lung cancer-related deaths compared with older men. A major limitation of this study was the potentially confounding effects of age and, in addition, the use of HRT in postmenopausal women was not examined.

More recent studies have examined the effects of HRT on survival. In a retrospective analysis of 429 women with lung cancer, overall survival was significantly higher in women with no HRT compared with patients who received HRT (79 vs 39 months; hazard ratio [HR]: 1.97). The survival benefit appeared to be more pronounced in women with a prior history of smoking [14]. In a *post-hoc* analysis of the Women's Health Initiative trial (a randomized double-blind placebo-controlled trial of 16,608 postmenopausal women comparing combined HRT with placebo), Chlebowski *et al.* found that more women died from lung cancer in the combined hormone therapy group than in the placebo group (HR: 1.71; 95% CI: 1.16–2.52; $p = 0.01$). These effects were more pronounced in women who developed non-small-cell lung cancer (NSCLC; HR: 1.87; 95% CI: 1.22–2.88; $p = 0.004$) [15]. Two other retrospective analyses have, however, failed to observe difference in lung cancer outcomes in women with lung cancer based on HRT use [16,17].

Laboratory evidence of the role of estrogen & progesterone in lung cancer

Estradiol (or E2) has been shown to promote the growth of both normal lung fibroblasts and lung cancer cells *in vitro* and *in vivo* [18,19]. E2 also increased secretion of hepatocyte growth factor (HGF), a potent mitogen, pro-motility and pro-invasive factor from normal lung fibroblasts and VEGF from lung cancer cells *in vitro* [19,20]. Conversely, siRNA-mediated knockdown of the estrogen receptors (ERs) significantly decreased the proliferation of NIH-H23 lung cancer cells *in vitro* [21].

The proliferative effect of estrogen in lung cancer cells is mediated through two main pathways – the genomic or ‘classical’ pathway involving primarily ER β and possibly ER α , and the non-genomic pathway involving both the ERs (ER α and β). ER α and ER β are encoded by two distinct genes, *ESR1* and *ESR2*, located on the chromosome loci 6q25.1 and 14q23.2, respectively. Both receptors are expressed as full-length proteins (molecular weight 66 and 65 kDa, respectively) and as several transcript variants [22]. While ER β is expressed as a full-length variant in both the normal lung (in the bronchial epithelium and fibroblasts) and in lung cancer cells, full-length ER α is either absent or is expressed at very low levels. The predominant form of ER α in both normal lungs and in lung cancer cells and tissues is believed to be the one lacking exon 4 (Δ exon 4), the exon encoding the nuclear localization signal located near the amino (NH₂)-terminus of the protein. As a result, ER α in the lungs is predominantly cytoplasmic in location. Indirect evidence for the presence of an NH₂-terminal truncated variant of ER α came from studies that reported an absence of ER α expression using antibodies raised against the NH₂-terminus of the protein (TABLE 2).

Other studies that employed antibodies recognizing the carboxyl terminus of ER α , however, noted that the receptor was expressed in NSCLC tissues and cell lines (albeit at levels nearly 40-fold lower than that in the breast cancer cells) but not in small-cell lung cancer (SCLC) tissues [19,23]. While most studies reported that the staining for ER α was localized to the cytoplasm, a few reported nuclear expression of the receptor [20,21]. By contrast, breast cancer tissues, where ER α is the predominant functioning ER, exhibit a strong nuclear expression of the receptor with the same antibody.

The ER-mediated signaling pathways are summarized in FIGURE 1. Signaling through the ERs is activated upon binding to their ligand, E2 (ER β). In addition to circulating estrogen and exogenous estrogen (from HRT), E2 is also produced locally by NSCLC cells. This is achieved by the enzyme aromatase whose expression is detectable in both NSCLC tissues and cell lines [20].

Estrogen receptors remain sequestered in the cytoplasm bound to an inhibitory complex comprising several proteins, including the heat-shock protein HSP90. Binding of ER β to estrogen releases it from this inhibitory complex. In the case of ER β , this is followed by dimerization and translocation of the receptor to the nucleus or into caveolae in the plasma membrane. Upon entering the nucleus, ER β binds to estrogen response elements located in the promoters of target genes, where it recruits coactivators such as GRIP1/TIF2, forming functional transcription complexes [24], or SRC-3 (AIB1) and MNAR/PELP3 (p160 coactivators) [21], which are thought to play a key role in modulating ER transcriptional activity [25].

The fate of ER α , however, is somewhat controversial. Marquez-Garban *et al.* observed that phosphorylated ER α (the active form of the receptor) is localized in the nucleus of tissues from NSCLC patients using immunohistochemical analysis. The phosphorylations occurred on two serine residues, S167 and S118, located in the hormone-independent transcription activation function domain (AF-1) of ER α near its NH₂-terminus [21]. Phosphorylation at S167 is mediated by Akt and has been suggested to be a mechanism for Akt-mediated resistance of breast cancer cells to tamoxifen [26]. S118 on the other hand may be a target of the transcription regulatory kinase cdk7 [27]. However, Hershberger and colleagues, in a separate study, did not detect any ER α expression in NSCLC cells by western blotting using a different antibody (sc-543) against the C-terminus of the receptor (Michigan Cancer Foundation [MCF]-7 breast cancer cells were used as positive controls) [24]. Two possible reasons could explain the failure of Hershberger's group to detect the transcript variant of ER α . One is that the antibody recognizes a 3D conformation (of full-length ER α), which is lost in the transcript variants. Second, and more plausibly, the transcript variant is expressed at such low levels that it is undetectable by western blotting. A study by Fasco and colleagues had found that Δ exon 7 was the most common transcript variant of ER α in lung cancer tissues, with Δ exon 3, Δ exon 4, Δ exon 5 and Δ exon 3 + 4 being far less common [28]. Of these, the Δ exon 4 variant, first described more than a decade ago in ZR 75-1 breast cancer cells [29], was the only one that could not enter the nucleus by itself (owing to lack of the nuclear localization signal). However, it could do so either as a homodimer with a molecule of full-length ER α , or as a heterodimer with ER β . In a study of cultured normal bronchial epithelial cells and lung fibroblasts, Stabile *et al.* had identified that the full-length ER α and the Δ exon 7 variants were the most common ER α transcripts expressed in lung cancer cells [19]. However, they failed to detect the full-length protein in western blotting using an antibody that detected full-length ER α in MCF-7 cells. The reason for this is unclear, and suggests that the question regarding the presence of ER α in lung cancer remains open.

In addition to their direct effects on transcription, the ERs can also modulate signaling by binding to cell surface proteins. Both full-length ER α and β have been shown to co-purify with EGF receptor (EGFR) and the caveolar proteins caveolin and flotillin in NIH-H23 NSCLC cells [20]. ER α also appeared as clusters on the plasma membrane of nonpermeabilized NIH-H23 cells, suggesting that the receptor possibly localizes in caveolin containing lipid rafts in the cell membrane. ER β , but not ER α , treatment of these cells also led to rapid activation of p44/p42 MAP kinase (ERK1/ERK2), followed by nuclear localization of the activated ERK1/2. The anchoring of the ER to the plasma membrane requires the palmitoylation of two cysteine residues (Cys-477 and Cys-530). A point mutation in any one of the two cysteines (Cys-477-Ser or Cys-530-Ser) blocked localization of ER α to the plasma membrane and reduce the levels of phosphorylated ERK1/2. Furthermore, treatment of the cells with a palmitoyl-transferase inhibitor decreased E2-induced cell proliferation in lung cancer cells [20]. Taken together, these observations suggest that ER α anchors to the cell membrane through palmitoylation of key cysteine residues and this is an important prerequisite for it to interact with cell surface receptors and activate growth-promoting pathways in lung cancer cells.

Detection of ERs (positive vs negative) has been evaluated for their prognostic significance in NSCLC. Kawai *et al.* observed that cytosolic expression of ER α was associated with a higher grade of the tumor (60, 84 and 74% positivity in well, moderately and poorly differentiated NSCLC, respectively) and poor overall survival (OS) in NSCLC patients on univariate analysis. ER β nuclear expression on the other hand did not correlate with grade but was associated with a significantly better OS. Furthermore, ER $\alpha^+\beta^-$ patients had a significantly worse OS (HR: 1.9; 95% CI: 1.1–3.4) than ER $\alpha^-\beta^+$ patients [23].

In an analysis of the role of ER β alone without consideration of ER α , Schwartz and colleagues reported that ER β was expressed in the nucleus in 61% and in the cytosol in 46% of NSCLC samples [30]. In comparison, 20% and 17% of adjacent normal lung tissue (normal bronchial and alveolar mucosa and peripheral normal lung tissue) also expressed ER β in the nucleus and cytosol, respectively. While ER β expression did not show any association with patient demographics, smoking history, menopausal status, stage, grade of tumor, history of HRT or OCs, there was a predilection for gender, with males being more commonly positive than females (70 vs 58%, respectively; $p = 0.09$). The gender difference was strongest for adenocarcinomas, with females having a 60% lower chance of being ER β^+ than males. In men, ER β^+ tumors were associated with better survival than ER β^- tumors (HR: 0.45; $p = 0.04$), whereas in women ER β positivity nonsignificantly increased their risk of dying ($p = 0.13$) [30].

Epigenetic changes in ER—Epigenetic changes, chiefly methylation of the CpG islands in the ER α , have been suggested to be a mechanism for regulating ER α expression in lung cancer. In one study, none of the ER α CpG islands were methylated in normal lung tissue [31]. By contrast, 11 out of 46 lung cancer tissues showed significant (>10%) methylation at the *NotI* restriction site in the ER promoter. While the prevalence of ER methylation in these tissues did not differ with gender, type or stage of lung cancer, patients with a history of smoking had a significantly lower incidence of ER promoter methylation (20%) than nonsmokers (36%). Since methylation leads to gene silencing, this may provide indirect evidence for the synergistic activity of cigarette smoke and the ER pathway in the development of lung cancer.

Methylation of the ER promoter was also evident in lung cancer cell lines, ranging from partial (H720, H1299 and OH1 cells) to complete (in H358 cells) methylation at the *NotI* site. ER methylation was also observed in 85% of lung tissue specimens in a spontaneous mouse model (A/J strain) of lung cancer between 18 and 24 months of age. 4-

(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a carcinogen found in smoke, induced the enzyme *Mtase* in type II pneumocytes in the mouse lung. These cells are considered to be precursors of lung adenocarcinoma in mice and this could at least partly explain the methylation at the ER promoter in lung cancer cells.

Possible interactions between testosterone & estrogen—While estrogen appears to be the main procarcinogenic hormone linked to lung cancer, observations in a mouse model suggest that other factors may also be important. Hammoud *et al.*, examining the effect of E2 on lung cancer development in a *Kras*^{G12D/+}*-TP53*^{-/-} murine lung cancer model, observed that ovariectomy reduced the number and volume of lung tumors in the female transgenic mice, an effect that was reversible by exogenous administration of E2 [32]. However, the protective effect of ovariectomy was only apparent in mice that were administered high doses of the adenovirus containing Cre recombinase (Adeno-Cre), which was needed to express *Kras*^{G12D} and knock out *TP53*.

Male transgenic mice, on the other hand, also showed an increase in tumorigenicity (size and volume) with administration of E2, but at a 50-fold lower dose of Adeno-Cre than that required in the female mice [32]. The significantly higher tumorigenic effect of E2 observed in the male transgenic mice suggests that androgens might potentiate the proliferative effects of estrogen. Another possibility could be the modulating effects of progesterone. While the role of progesterone is discussed in detail later, progesterone is metabolized to testosterone in the ovaries and adrenal glands in women [33], and it is possible that loss of progesterone contributed to the lower tumorigenicity of E2 in the ovariectomized mice. Circulating high levels of testosterone (in men and postmenopausal women taking progesterone-containing HRT) could thus contribute at least in part to the development and/or progression of lung cancer.

Role of progesterone—Progesterone receptors (PRs), although not as well studied in lung cancer, have nonetheless been shown to be expressed in NSCLC [34-37]. Like estrogen, progesterone is also synthesized *in situ* by NSCLC cells [36]. While PR expression did not correlate with age, menopausal state, or ER or p53 positivity of NSCLC tissues, it was more commonly positive in females, early-stage disease and in poorly differentiated NSCLC. Furthermore, PR positivity was an independent predictor of better OS on multivariate analysis. Progesterone also inhibited the growth of subcutaneously implanted lung cancer cells in nude mice, associated with a decrease in the expression of Ki-67, cyclin A and E and an increase in p21 and p27 levels [36].

Another study reported that 44% of lung cancers expressed PR, and while the incidence of positivity was not as high as the earlier study, PR was still more commonly positive in adenocarcinomas (53%; n = 17) than in SCCs (39%; n = 18) or large-cell carcinomas (27%; n = 11). PR mRNA was also expressed in six out of 11 SCLC and 13 out of 17 NSCLC cell lines by RT-PCR [35]. However, a more recent investigation contradicts the findings of both these studies. In contrast to the study by Ishibashi and coworkers [36], Raso *et al.* failed to detect any association between PR positivity and overall survival [37]. Also in contrast to the Kaiser study [35], they found that squamous cell carcinomas (SCCs) showed a higher frequency of PR expression (70%) than adenocarcinomas (58%).

Role of aromatase—As mentioned earlier, aromatase is responsible for a key step in estrogen biosynthesis. In one study, aromatase activity (measured by the amount of E2 produced after treatment of cells with testosterone) was significantly higher in lung cancer tissues (adenocarcinoma and SCC) than in the adjacent normal lung tissue [18]. The same study also noted that aromatase was expressed by adenocarcinomas (88%; n = 40), adenosquamous carcinomas (100%; n = 1), SCCs (87%; n = 8) and bronchoalveolar

carcinomas (75%; n = 4) alike. Aromatase expression in these tissues was localized primarily to the tumor epithelial cells with no staining in the interstitial tissue. Weak positivity, however, was also noted in the adjacent normal bronchioles and macrophages. Interestingly, there was no difference in aromatase positivity (defined as >15% cells positive for the enzyme) between males and females (86 vs 88% positive, respectively).

In a study of 422 NSCLC specimens, Mah *et al.* showed that aromatase was localized in the cytoplasm and the staining intensity as determined by immunohistochemistry correlated well with aromatase enzyme activity as assessed by a radioassay [38]. Aromatase staining was seen in all tumor subtypes as well as in the non-neoplastic bronchial epithelium. They also found that lower levels of aromatase predicted a better survival in women above 65 years of age. In addition, for women with no history of smoking, a lower aromatase level was a strong predictor of longer survival.

Preclinical evidence of anti-estrogen therapy in lung cancer

Estrogen receptors and aromatase have also been explored as targets for lung cancer therapy. In one study, fulvestrant, a competitive inhibitor of estrogen binding at the ER, significantly reduced tumor volume of NCI-H23 NSCLC cells implanted sub cutaneously into ovariectomized nude mice. Significantly, erlotinib (an EGFR tyrosine kinase inhibitor) when given alone did not have any significant effect on *in vivo* growth of NCI-H23 cells. However, a combination of fulvestrant with erlotinib was more effective than fulvestrant alone in reducing tumor volume [21].

A similar synergistic growth-inhibitory effect was noted *in vitro* (in NIH-H23 cells) when fulvestrant was combined with gefitinib, another EGFR antagonist. Further, the combination also reduced basal secretion of VEGF, while fulvestrant blocked ER β -induced VEGF secretion from lung cancer cells *in vitro* [20]. Inhibitors of aromatase (e.g., anastrozole) have also demonstrated significant growth inhibitory activity (>90%) against A549 lung cancer cells (ER β ⁺ aromatase⁺) both *in vitro* and upon implantation in nude ovariectomized mice [18].

Treatment of lung NSCLC xenografts *in vivo* with exemestane (an aromatase inhibitor) alone or in combination with cisplatin caused significant reduction in tumor progression compared with paired controls. Lending credence to the observation that lung cancer progression is governed by complex interactions between ER and growth factor pathways, a combination of fulvestrant and the multi-targeted growth factor receptor inhibitor, vandetanib, inhibited tumor growth in xenograft models more effectively than either treatment alone [39].

Clinical evidence of estrogen inhibitors in lung cancer

Given this evidence on the role of ERs in the pathogenesis of lung cancer, it is not surprising that clinical trials have evaluated the role of various ER blockers in lung cancer. Two small Phase II trials from Taiwan evaluated the combination of tamoxifen with ifosfamide, epirubicin and cisplatin in patients with relapsed NSCLC [40,41]. They found response rates ranging from 20 to 47%, with progression-free survival (PFS) and OS of 4.9–6 and 7.7–12 months, respectively. While this may not have been the most suitable design to test for the role of the ER pathway, given that tamoxifen is a partial agonist/antagonist of estrogen, these results in the relapsed setting are worth considering.

A pilot study examining the efficacy of the combination of fulvestrant and gefitinib in 22 postmenopausal patients with advanced NSCLC (eight adenocarcinoma, six NSCLC not otherwise specified, four SCC and four bronchoalveolar carcinoma patients) revealed that patients who had at least 60% ER β nuclear staining by immunohistochemistry (n = 8) had a

median OS of 65.5 weeks. In comparison, those with less than 60% ER β staining (n = 5) had a median survival of 21 weeks [42]. In a recent report, Collins *et al.* described the case of a female patient diagnosed with ER $^{+}$, PR $^{-}$, TTF-1 $^{+}$, EGFR $^{+}$ stage IV adenocarcinoma, who responded significantly (improved symptoms with 58% decrease in tumor size) to exemestane after progressing while being treated with a combination of chemotherapy (cisplatin and vinorelbine), immunotherapy (cetuximab) and bisphosphonates [43].

In a Phase III study in patients with limited-stage SCLC, the Cancer and Leukemia Group B randomized patients to standard cisplatin–etoposide with concurrent thoracic radiation or the same regimen with the addition of tamoxifen [44]. The addition of tamoxifen had no effect on response rates, PFS or OS. This has somewhat dampened the enthusiasm for the use of tamoxifen in patients with lung cancer in general and SCLC in particular.

Expert commentary

The role of estrogens in lung carcinogenesis cannot be disputed based on the evidence presented, the disappointing results of the tamoxifen clinical trials notwithstanding. HRT does appear to have detrimental effects, not only on the outcomes from lung cancer, but also on its incidence. ERs are expressed in NSCLC at a level similar to that in the normal bronchial epithelium and fibroblasts. ER β is the predominant receptor that appears to mediate the proliferative effects of E2 on lung cancer cells. While it is unclear whether estrogens alone can initiate development of lung cancer, they do appear to promote progression of established lung cancer. The ER competitive antagonist fulvestrant inhibits E2-induced cell proliferation while aromatase inhibitors appear to be effective in inhibiting lung cancer proliferation, even in the absence of exogenous estrogen. The underlying mechanism appears to be the inhibition of E2 produced locally by the NSCLC cells. Furthermore, estrogen interacts with and activates EGFR-induced downstream signaling. Combinations of anti-estrogens with EGFR antagonists appear promising. Progesterone and androgens also appear to have a role in the progression of lung cancer; however, their effects and the mechanisms underlying them need to be elucidated further.

Five-year view

As evidence on the role of the ER on lung carcinogenesis rapidly accumulates, the exact role of the two subtypes ER α and ER β in the proliferation of lung cancer cells, especially in conjunction with EGFR, will be identified. This should lead to better experimental and clinical models for the combined inhibition of EGFR and ER-mediated pathways. The effects of ER inhibition on the cytotoxicity of chemotherapeutic agents will be better understood, leading to the development of clinical trials exploiting this approach. This may also shed further light on the development of lung cancer in never smokers or former light smokers.

Key issues

- A much smaller proportion of cases of lung cancer in women are attributable to smoking compared with in men.
- Women with lung cancer who have taken hormone-replacement therapy have a worse outcome compared with those who have not.
- While both estrogen receptors ER α and ER β have been identified in lung cancer cells, ER β is predominantly seen.
- *In vitro* studies suggest that ER may act in conjunction with EGF receptor in lung carcinogenesis. Combinations of estrogen antagonists (exemestane,

fulvestrant) with anti-EGF receptor therapies (gefitinib, vandetanib) have a synergistic effect and inhibit growth of lung cancer cells *in vitro* more than either agent alone.

- Clinical trials exploiting the estrogen receptor pathways in lung cancer are currently underway.

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Figure 1. Estrogen receptor-mediated signaling in lung cancer

Lung cancer cells predominantly express ERβ. The full-length ERα, the primary functional receptor in breast cancer cells, is not expressed in most lung cancer cells and tissues. Instead, they express a variant of ERα that lacks the exon 4 at the amino terminus (which contains the nuclear localization signal). As a result, ERα is expressed in the cytoplasm of non-small-cell lung cancer (NSCLC) cells and tissues, while ERβ predominantly localizes to the cytoplasm and is believed to mediate the transcriptional effects of estrogen in NSCLC. Estrogen (17-β estradiol or E2β) present in the extracellular space diffuses through the cell membrane and binds to cytosolic ERs, α or β. The ERs in the cytoplasm are normally kept inactive by binding of a complex containing HSP90. Binding with estrogen causes the inhibitory complex to dissociate, thereby activating the receptor. The ligand-bound receptor then either dimerizes and is imported into the nucleus (classical pathway [I] mediated by ERβ) or translocates to the cell membrane where it associates with receptor tyrosine kinases like the EGFR (alternate pathway [II] mediated by either ERα and/or ERβ). The ER proteins have been shown to colocalize in caveolae, which are plasma membrane-associated lipid rafts that provide the appropriate environment for interaction with cell surface receptors like EGFR. This interaction with EGFR forms the basis of employing EGFR tyrosine kinase inhibitors in combination with anti-estrogens (e.g., fulvestrant, a competitive antagonist of estrogen binding to the ER) for the treatment of lung cancer. In the case of ERα, palmitoylation of a cysteine residue (Cys477) is crucial for its localization to the plasma

membrane. The activation of EGFR by ER α or β (bound to the ligand E2 β) leads to phosphorylation of the adaptor protein Shc, which in turn associates with the GRB2–SOS complex, activating the Ras/Raf/MAPK pathway. The activated ERK (p44/p42 MAPK) then migrates to the nucleus where it activates the transcription of genes that promote proliferation and invasion of NSCLC cells. In addition, the ligand-bound ER β receptor, upon translocating to the nucleus, binds directly to gene promoters containing the ERE where it recruits the p160 coactivator GRIP1/TIF2 and thus activates genes that promote cell cycle progression (Id-2 and cyclin D1) and therefore induces proliferation of NSCLC cells. EGFR: EGF receptor; ER: Estrogen receptor; ERE: Estrogen response element; ERK: Extracellular signal-regulated kinase; GRB: Growth factor receptor-bound protein; GRIP: Glutamate receptor interacting protein; HSP: Heat-shock protein; SOS: Son of sevenless.

Table 1
Epidemiologic studies of hormone-replacement therapy and incidence of lung cancer

Author (year)	Type of study	Participants (n)	Conclusions	Ref.
Schabath <i>et al.</i> (2004)	Case-control	499 cases; 519 healthy age-matched controls	HRT: 34% reduced risk of lung cancer ERT: 35% reduction Combination therapy: 39% reduction HRT: 41% reduced risk in current smokers	[8]
Ramnath <i>et al.</i> (2007)	Case-control	595 cases; 1195 controls	HRT: 33% reduced risk of lung cancer, more pronounced in former smokers and women with normal-to-low BMI	[9]
Schwartz <i>et al.</i> (2007)	Case-control	488 cases; 498 controls	Increased duration of HRT use in quartiles associated with 12% decreased risk of NSCLC in postmenopausal women	[10]
Chen <i>et al.</i> (2007)	Case-control	826 cases; 531 healthy controls	HRT: 30% reduced risk of lung cancer	[11]
Blackman <i>et al.</i> (2002)	Case-control	662 cases; 4671 controls	No effect (either increase or decrease) of ERT on incidence of lung cancer	[45]
Kreuzer <i>et al.</i> (2003)	Case-control	811 cases; 912 controls	OC: 31% reduction in lung cancer risk HRT: 17% reduced risk, 41% after ≥ 7 years of usage Reduction in lung cancer risk associated with exogenous hormones primarily seen among smokers	[46]
Taioli <i>et al.</i> (1994)	Case-control	180 cases; 303 controls	Increased risk of adenocarcinoma (OR: 1.7) Significant synergy between smoking and HRT for development of lung cancer (OR: 32.4) No effect of HRT on nonsmokers (OR: 1.0)	[6]
Adami <i>et al.</i> (1989)	Cohort	Population-based cohort of 23,244 women	HRT: Nonsignificant increase in the risk of lung cancer (RR: 1.3; 95% CI: 0.9–1.7)	[7]
Smith <i>et al.</i> (2009)	Cohort	2861 women	HRT: 58% increased risk in women >55 years old	[47]
Slatore <i>et al.</i> (2010)	Cohort	36,588 peri- and postmenopausal women aged 50–76 years	48% increased risk of lung cancer with ≥ 10 years of combined HRT No association with duration of ERT	[48]
Chlebowski <i>et al.</i> (2009)	Post-hoc analysis of RCT	8506 women assigned to combined HRT; 8102 to placebo	23% non-significant increased risk of developing NSCLC	[15]

ERT: Estrogen-replacement therapy; HRT: Hormone-replacement therapy; NSCLC: Non-small-cell lung cancer; OC: Oral contraceptives; OR: Odds ratio; RCT: Randomized controlled trial; RR: Relative risk.

Table 2
Summary of antibodies against estrogen receptors and positivity for estrogen receptors in lung cancer tissues and cell lines

Author (year)	Antibody (N- or C-terminus)	Estrogen receptor positivity	Ref.
Kawai <i>et al.</i> (2005)	ERα: HC-20 (C-terminus) ERβ: H-150(1:10)	73% in LC (cytoplasm) 51% in LC (nucleus)	[23]
Ishibashi <i>et al.</i> (2005)	ERα: 6F11 (N-terminus) ERβ: 14C8	38% (nucleus) 34% (nucleus)	[36]
Marquez-Garban <i>et al.</i> (2007)	ERα: HC-20 (C-terminus) p-Ser118 ERα: 16J4 p-Ser167 ERα: 314778 ERβ: not named	Nuclear: 49% (female) vs 35% (male); extranuclear: 78% (female) vs 70% (male) Not mentioned 67% (nuclear) Nuclear: 51% (female) vs 55% (male); extranuclear: 64% (female) vs 80% (male)	[21]
Schwartz <i>et al.</i> (2005)	ERα: 1D5 (N-terminus) ERα: 6F11 (N-terminus) ERβ: 14C8 (N-terminus) ERβ: PAI-313 (C-terminus) ERβ: MCA1974S (C-terminus)	0% Nuclear: 61% (LC) vs 20% (normal lung); cytoplasm: 46% (LC) vs 17% (normal lung)	[30]
Di Nunno <i>et al.</i> (2000)	ERα: 1D5 (N-terminus)	0%	[49]
Kaiser <i>et al.</i> (1996)	ER: ERICA (not mentioned)	8% of lung cancers (n = 52) were positive. 1/18 SCCs (5.6%), 2/17 adenocarcinomas (12%), 0/17 LCCs (0%), 0/3 SCLCs (0%), 0/1 BACs (0%) were positive	[35]
Weinberg <i>et al.</i> (2005)	ERα: 1D5 (N-terminus) ERβ: D7N	All NSCLC cell lines tested (H23, H2122, H1650, A549, A427, A125) express a 67-kDa and an 80-kDa form of ERα (40-fold lower than MCF-7) by western blotting All NSCLC cell lines tested above expressed a single band of ERβ (59 kDa)	[18]
Stabile <i>et al.</i> (2002)	ERα: HC-20 (C-terminus) ERβ: recognizes amino acids 512–530 at C-terminus	None of the NSCLC cells expressed the 67 kDa (full-length) ERα expressed by MCF-7 cells by western blotting. All of them however expressed a 54-kDa and 80-kDa band that were also present in MCF-7 cells. ERα was expressed in the cytoplasm of squamous lung dysplasia and SCC tissues by IHC. ERα was also expressed by these tissues by western blotting 59-kDa band corresponding to full-length ERβ was detected in all LC cells ERβ was expressed in the nucleus of squamous lung dysplasia and SCC tissues by IHC. ERβ was also expressed by these tissues by western blotting	[19]
Hershberger <i>et al.</i> (2005)	ERα: sc-544 (hinge region) ERα: sc-543 (C-terminus) ERα: sc-7207 (N-terminus) ERβ: sc-8974	No ERα detected in normal lung fibroblasts or in LC cell lines by western blotting ERβ was expressed by all normal lung fibroblasts and LC cells by western blotting	[24]

Author (year)	Antibody (N- or C-terminus)	Estrogen receptor positivity	Ref.
Radzikowska <i>et al.</i> (2002)	ER α : 6F11 (N-terminus)	Weak nuclear expression in one female with adenocarcinoma (5%, n = 18) with clone 6F11 and in one male with	[50]
	ER α : 1D5 (N-terminus)	SCC (7%, n = 14) with clone 1D5	

BAC: Bronchoalveolar carcinoma; ER: Estrogen receptor; IHC: Immunohistochemistry; LC: Lung cancer; LCC: Large cell carcinoma; MCF: Michigan Cancer Foundation; NSCLC: Non-small-cell lung cancer; SCC: Squamous cell carcinoma; SCLC: Small-cell lung cancer.