Nuclear Estrogen Receptor β in Lung Cancer: Expression and Survival Differences by Sex

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Abstract

Purpose: A role for estrogens in determining lung cancer risk and prognosis is suggested by reported sex differences in susceptibility and survival. Archival lung tissue was evaluated for the presence of nuclear estrogen receptor (ER)-α and ER-β and the relationship between ER status, subject characteristics, and survival.

Experimental Design: Paraffin-embedded lung tumor samples were obtained from 214 women and 64 men from two population-based, case-control studies as were 10 normal lung autopsy samples from patients without cancer. Nuclear ER-α and ER-β expression was determined by immunohistochemistry. Logistic regression was used to identify factors associated with ER positivity and Cox proportional hazards models were used to measure survival differences by ER status.

Results: Neither tumor (0 of 94) nor normal (0 of 10) lung tissue stained positive for ER-α. Nuclear ER-β positivity was present in 61% of tumor tissue samples (170 of 278; 70.3% in men and 58.3% in women) and 20% of normal tissue samples (2 of 10; $P = 0.01$). In multivariate analyses, females were 46% less likely to have ER-β− positive tumors than males (odds ratio, 0.54; 95% confidence interval, 0.27-1.08). This relationship was stronger and statistically significant in adenocarcinomas (odds ratio, 0.40; 95% confidence interval, 0.18-0.89). Women with ER-β− positive tumors had a nonsignificant 73% ($P = 0.1$) increase in mortality, whereas men with ER-β− positive tumors had a significant 55% ($P = 0.04$) reduction in mortality compared with those with ER-β− negative tumors.

Conclusions: This study suggests differential expression by sex and influence on survival in men of nuclear ER-β in lung cancer, particularly in adenocarcinomas.

In the United States, lung cancer is the second most common cancer among both men and women and is the leading cause of cancer death in both sexes (1). A number of studies report that women are more susceptible, dose for dose, to the carcinogenic effects of cigarette smoke than men (2, 3). Women have been reported to have higher levels of polycyclic aromatic hydrocarbon-DNA adducts than men at any given level of smoking (4). Smoking females have significantly higher levels of expression of the gene encoding CYP1A1, a central enzyme in the metabolic activation of polycyclic aromatic hydrocarbons (4, 5). It has also been shown that G:C to T:A transversions in *p53* are more common among females with lung cancer than males (6). One study also reported that the proportion of nonsmoking lung cancer cases in women was double that in men, suggesting that even nonsmoking women may be more susceptible to lung carcinogens than nonsmoking men (2).

The reported sex difference in susceptibility suggests a role for hormones in determining lung cancer risk. Both exogenous estrogens [i.e., hormone replacement therapy (HRT) and oral contraceptives] and endogenous hormone levels (i.e., age at menopause) may contribute to the development of lung cancer (7–10). Early age at menopause has been associated with decreased risk of adenocarcinoma of the lung, whereas use of estrogen replacement therapy has been associated with both increased (7) and decreased lung cancer risk (11). A slightly increased risk of lung cancer after estrogen treatment (relative risk, 1.3) has been reported (9), whereas decreased risk has been associated with ≥2 years use of oral contraceptives [odds ratio (OR), 0.6; 95% confidence interval (95% CI), 0.2-0.8; ref. 8]. In China, where the prevalence of smoking among women is low, lung cancer risk was found to be higher among women with a late menopause (10). These findings support the hypothesis that circulating hormones may play a role in the development of lung cancer in women.

Estrogens could act as tumor promoters through a receptor-mediated mechanism to increase cell proliferation (12). Two

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subtypes of estrogen receptor (ER) have been described, ER-α and ER-β, both of which have a high affinity for estradiol. The tissue distribution of these two receptors varies and includes a number of estrogen "target" and "nontarget" tissues (13). ER expression has been detected in normal lung and lung tumor tissue (14–19); however, studies completed before ER-β was identified in 1996 (20) could not distinguish between ER-α and ER-β. Only a few studies have evaluated ER-β expression in the lung (15–19). No studies to date have used population-based case selection and few have evaluated differences in ER expression by subject characteristics or its role in survival. In this study, archival lung tissue from two population-based, case-control studies and normal lung tissue from autopsy were evaluated for the presence of nuclear ER-α and ER-β and the relationship between ER status, subject characteristics, and survival.

Materials and Methods

Study population. Subjects were selected through two population-based, case-control studies of lung cancer in the metropolitan-Detroit tricounty area: One study is enrolling women ages 18 to 74 diagnosed with primary lung cancer from 2001 forward, whereas the second study enrolled men and women ages 18 to 49 diagnosed with primary lung cancer 1990 to 2004. Both study protocols were approved by the institutional review board and each subject provided informed consent for interview and biospecimen collection. Cases were ascertained through the Metropolitan Detroit Cancer Surveillance System, a population-based cancer registry participating in the Surveillance, Epidemiology, and End Results program of the National Cancer Institute. Paraffin-embedded lung tumor tissue was obtained for 278 individuals in these studies; 214 samples were from women and 64 samples were from men. Ten normal autopsy lung tissue samples from patients with no history of cancer (six men, four women) were obtained from one hospital pathology department. All samples were reviewed by a single pathologist (F. Lonardo) to verify diagnosis of lung cancer.

Subject characteristics were acquired through the Metropolitan Detroit Cancer Surveillance System Surveillance, Epidemiology, and End Results registry (i.e., age at diagnosis, sex, stage at diagnosis, and survival) and personal interviews (i.e., race, smoking status, medical history, oral contraceptive use, HRT use, and menopausal status). Race was categorized as White and non-White, which included 60 African-Americans, 5 Hispanics, 2 American Indian/Alaska Natives, 1 Middle-Easterner, and 2 others. Lung cancers were classified histologically following WHO guidelines (21), as adenocarcinoma, bronchioloalveolar carcinoma, squamous cell carcinoma, adenosquamous carcinoma, large cell carcinoma, or small cell carcinoma. Cases of non–small cell carcinoma that were too poorly differentiated to be included in more specific subgroups, and did not fit in the category of large cell undifferentiated carcinoma, were classified as non–small cell carcinoma, not otherwise specified. Stage at diagnosis was categorized as regional or distant versus local stage based on Surveillance, Epidemiology, and End Results guidelines. Menopausal status was categorized as premenopausal and postmenopausal and other variables were dichotomized as ever/never exposed.

Immunohistochemistry. Five-micrometer sections of lung tumor tissue were mounted on positively charged slides and dried for 1 hour at 60°C for immunohistochemistry assays for ER-α and ER-β expression. Heat-induced epitope retrieval was carried out using citrate buffer (pH 6.0). Slides were treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Nonimmune serum was used to block nonspecific staining. Monoclonal antibodies from two different clones were used to test for ER-α expression: clone 1D5 (DAKO Corp., Carpinteria, CA), which is raised against the NH_2-terminal domain (A/B region) of the receptor molecule, and clone 6F11 (Novocastra Labs), which is raised against the full-length form of the receptor molecule. Both antibodies were diluted 1:50, then incubated for 2 hours at room temperature. Biotinylated secondary antimouse antibody (1:100) was applied to the sections for 20 minutes. Known ER-α–positive breast tissue was used as a positive control. A negative control was created by substituting primary antibody with normal serum.

Three primary antibodies were used to test for ER-β on control tissue to determine which gave better staining results: ER-β-14C8 (Genetex, San Antonio, TX), which is raised against a recombinant fusion protein encoding amino acids 1 to 153, ER-β-PAI-313 (Affinity Bioreagents, Golden, CO), which is raised against a synthetic peptide corresponding to the COOH-terminal amino acid residues 467 to 485 of human ER-β and ER-β–MCA1974S (Serotec, Oxford, United Kingdom), which is raised against the COOH terminus of the β-1 isoform. The controls included benign hyperplastic prostate tissue (Fig. 1A), breast tissue, and granulosa cell tumor of the ovary. Serial dilutions of the ER-β-14C8 gave no positive staining. The ER-β-PAI-313 antibody used a 1:50 dilution overnight at 3°C and resulted in positively stained controls but results were not consistent and there was much background staining. The ER-β MCA1974S used a 1:10 dilution overnight at 4°C. Using this approach, control tissue stained in a consistent, clear manner. This antibody was then used to test the 278 samples for ER-β expression. After the slides were treated with primary antibody, they were incubated overnight at 4°C. A biotinylated secondary antimouse antibody (1:100) was then applied.

All immunohistochemistry assays used an ABC detection method (Vector Labs, Burlingame, CA). Immunohistochemistry was scored assessing separately the extent of positivity, graded by determining the percentage of positive tumor cells (<10% focal, 11-50% moderate, and 51-100% diffuse) and their staining intensity (1+ weak, 2+ moderate, and 3+ strong). Both nuclear and cytoplasmic staining was noted. Cases were scored as negative when they showed no visible nuclear staining, cytoplasmic staining only, or focal/weak (1+) nuclear staining. Positive results included samples with at least weak (1+) staining in ≥10% of tumor cells (Fig. 1B-D). An additional scoring method was also used in the tumor tissue. In the second approach, a positive result was scored as anything over 0% nuclear staining or any cytoplasmic staining. All tables use the first, more conservative, scoring criteria for positivity. Mention is made in the text about results using the looser criteria. The staining pattern in adjacent normal lung was studied in all tumor samples where there was a sufficient amount of adjacent normal lung tissue as well as in 10 cases of normal lung obtained at autopsy. The pathologist was blinded to all subject characteristics and survival status.

Statistical analysis. Statistical analyses were done using SAS V8.02 (SAS Institute, Cary, NC). Student's t tests were used for comparison of means, whereas χ^2 tests and Fisher's exact tests (when cell size was <5) were used for comparison of proportions. Multivariate models were constructed using logistic regression and included age, race, sex, histology, and other variables significant in a univariate analysis at $P < 0.1$, with ER-β positivity (yes/no) as the outcome of interest. OR and 95% CI estimates were generated from the coefficients in the logistic regression models.

Survival analysis was done using a Cox proportional hazards model (22). Subjects with <1 month of follow-up were excluded from analysis as were subjects diagnosed within 12 months of analysis to allow for at least 1 year of follow-up data, leaving 250 subjects for analysis. Five-year follow-up was set as the maximum follow-up time. Multivariate models included age, race, sex, stage, histology, and other variables significant in a univariate analysis at $P < 0.1$. Hazard ratios (HR) and 95% CIs were reported from maximum likelihood estimates. Cox regression plots, using S-Plus v.6.1 (Insightful Corporation, Seattle, WA), were used to graphically illustrate survival.

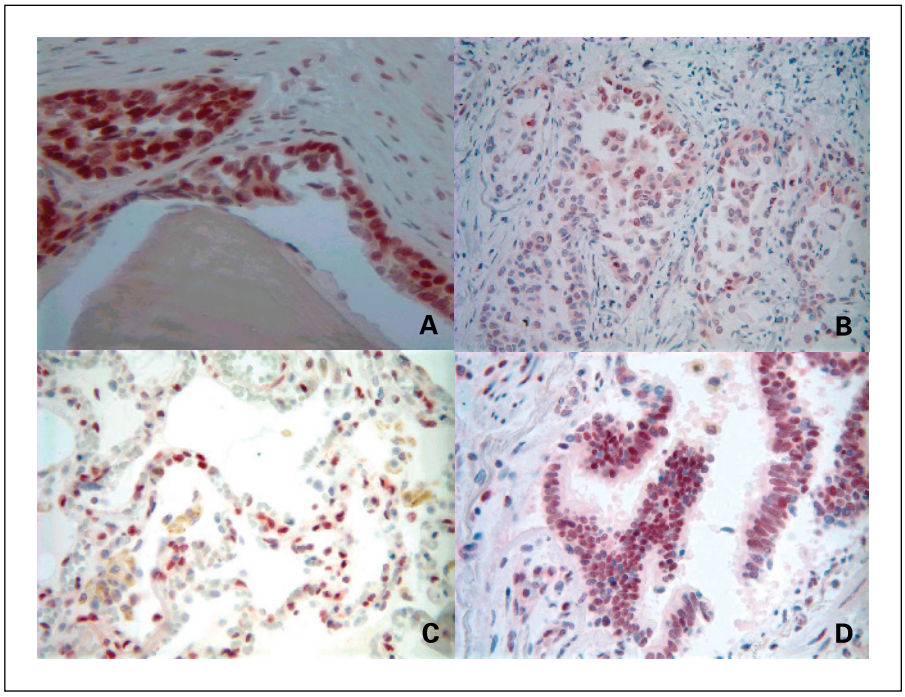

Fig. 1. Immunohistochemical staining of ER-β in human tissues. *A,* ER-β– positive staining in benign prostate hyperplastic tissue (control); *B,* ER-β– positive staining in adenocarcinoma of the lung; *C,* ER-β– positive staining in type II pneumocytes in lung tumor tissue; *D,* ER-β– positive staining in normal lung bronchus tissue.

Fifty-three cases reported a previous cancer diagnosis. All analyses were repeated excluding these cases. Analyses were also repeated only for cases with regional/distant disease, adenocarcinoma of the lung, and for an age-matched (5-year categories), stage-matched, and histology-matched subset of male and female cases. Additional analyses were conducted in women after stratification by menopausal status.

Results

Subject characteristics are listed in Table 1. Twenty-five percent of cases were non-White and 23% male, with a mean age of diagnosis of 53 years reflecting the nature of the studies, one of which focused on early age of onset lung cancer whereas the other included only women. Eighty-five percent of cases had a diagnosis of adenocarcinoma, bronchiolo-alveolar carcinoma, or adenosquamous carcinoma of the lung. None of the initial 94 subjects and none of the normal lung tissue samples (0 of 10) stained positive for ER-α; therefore, ER-α staining was discontinued.

Using the more conservative scoring criteria described, ER-β positivity was observed in 61% of tumor tissue samples (170 of 278) and in 20% of normal tissue samples (2 of 10; $P = 0.01$). ER-β positivity was also observed in 61.5% of tumor tissue samples from individuals without a previous cancer diagnosis and 59.8% of adenocarcinomas. Overall, cytoplasmic staining was detected in 46% of samples with nuclear staining and in 17% of samples without nuclear staining. Using the looser criteria for scoring positivity, 77.7% of tumor samples exhibited ER-β expression. No ER-β controls exhibited background staining.

Review of paired normal and tumor tissue was difficult because of the lack of adjacent normal tissue in most blocks. Among tumor samples that had sufficient adjacent normal tissue for analysis, when the tumor was ER-β positive then staining in normal structures was also observed [i.e., normal bronchi (82%; 62 of 76) and peripheral normal bronchiolar and alveolar mucosa (22%; 18 of 81) exhibited positivity when the tumor was positive]. Thirteen percent (14 of 110) of samples exhibited positivity across all paired tumor tissue, bronchial mucosa, and peripheral normal lung tissue. Positive tumor tissue was more likely to have detectable levels of positive peripheral normal mucosa ($P = 0.05$) and bronchial mucosa ($P = 0.08$), as well as more intense staining in peripheral mucosa ($P = 0.08$) and bronchial mucosa ($P = 0.02$) than ER-β–negative tumor tissue.

Subject characteristics by ER-β status are shown in Table 1. Males were more likely to have ER-β–positive tumors than females (70.3% of males versus 58.4% of females; $P = 0.09$) as were subjects with a history of chronic obstructive pulmonary disease (COPD; $P = 0.09$), although neither of these findings were statistically significant. There were no significant differences in ER-β status by smoking status, pack-years, use of HRT, use of oral contraceptives, menopausal status, histology, or stage at diagnosis (Table 1). Because of the eligibility criteria for entry into the studies included, women in this study were older (mean age 55.3 versus 44.1 years in men; $P < 0.001$), more likely to have adenocarcinomas ($P < 0.01$), and had earlier stage disease ($P < 0.01$) than men. None of these variables, however, were associated with nuclear ER-β expression and all were included in the multivariate analyses. There were also no significant differences in ER-β status by number of pregnancies, number of births, age at menarche, or age at menopause (data not shown). Results were essentially the same when analyses were restricted to adenocarcinomas, when individuals with a previous cancer diagnosis were excluded, when the matched subset was used, or when looser criteria for scoring was used. There were no significant trends with percentage of positive cells and subject characteristics (data not shown).

Multivariate logistic regression was done to identify potential factors associated with nuclear ER-β positivity. After adjusting

for race, age at diagnosis, stage at diagnosis, histology, smoking status, pack-years, and history of COPD, females were 46% less likely than males to have an ER-β–positive tumor (OR, 0.54; 95% CI, 0.27-1.08) although this finding was not statistically significant (Table 2). The relationship between nuclear ER-β positivity and sex was stronger and statistically significant in the analysis restricted to adenocarcinomas, with females having a significant 60% decreased likelihood of having an ER-β–positive tumor than males (OR, 0.40; 95% CI, 0.18-0.89; Table 2). In analyses limited to those without a previous cancer diagnosis, nuclear ER-β positivity was significantly associated with sex (OR, 0.45; 95% CI, 0.21-0.96). Similar, statistically significant, findings were seen in the matched subset (OR, 0.42; 95% CI, 0.18-0.97) and in the total sample using the looser scoring criteria (OR, 0.41; 95% CI, 0.18-0.95). ER-β positivity was not associated with race, age, stage at diagnosis, histology, smoking status, or pack-years of exposure in all subjects combined or in subset analyses. A history of COPD was reported 2.7 times more often in individuals with ER-β–positive tumors (95% CI, 0.83-8.51), although this finding was not statistically significant. The relationship with COPD was strongest among subjects with an adenocarcinoma diagnosis (OR, 3.76; 95% CI, 1.00-14.1). There was no association between nuclear ER-β positivity and oral contraceptive use, HRT use, menopausal status, age at menarche, or age at menopause in women in multivariate analyses.

The mean length of follow-up for subjects included in the survival analysis was 18.8 months (SD ± 13.2 months); the median was 16 months. The overall 1-year survival rate was 74.1% (160 of 216), whereas the 3-year survival rate was 21.3% (29 of 136). The effects of nuclear ER-β positivity on overall survival in a Cox proportional hazards model are summarized

Table 1. Subject characteristics and ER-β status

Characteristic	Total (n = 278)	ER-β positive (n = 170)	ER-β negative (n = 108)	P
	n* (%)	n* (%)	n* (%)	
Age, mean ± SD	52.7 ± 11.4	52.3 ± 11.0	53.3 ± 12.0	0.46
Sex				
Female	214 (77.0)	125 (58.4)	89 (41.6)	
Male	64 (23.0)	45 (70.3)	19 (29.7)	0.09
Race				
White	208 (74.8)	127 (61.1)	81 (38.9)	
Other	70 (25.3)	43 (61.4)	27 (38.6)	0.96
Smoking status				
Ever	247 (88.9)	154 (62.3)	93 (37.6)	
Never	30 (10.8)	16 (53.3)	14 (46.7)	0.34
Pack-years, mean ± SD	42.6 ± 26.4	42.9 ± 26.1	42.0 ± 27.1	0.80
History of COPD				
Ever	26 (90.6)	20 (76.9)	6 (23.1)	
Never	249 (9.4)	149 (59.8)	100 (40.2)	0.09
Oral contraceptive use (n = 214 women)				
Ever	144 (67.3)	81 (56.3)	63 (43.8)	
Never	50 (23.4)	31 (62.0)	19 (38.0)	0.48
HRT use (n = 214 women)				
Ever	85 (39.7)	46 (54.1)	39 (45.9)	
Never	109 (50.9)	66 (60.6)	43 (39.4)	0.37
Menopause status (n = 214 women)				
Postmenopause	164 (76.6)	95 (57.9)	69 (42.1)	
Premenopause	30 (14.0)	18 (60.0)	12 (40.0)	0.83
Histology				
Adenocarcinoma	216 (77.7)	128 (59.3)	88 (40.7)	
Bronchiolo-alveolar carcinoma	16 (5.8)	9 (56.3)	7 (43.8)	
Adenosquamous carcinoma	4 (1.4)	4 (100)	0 (0)	
Large cell carcinoma	9 (3.2)	5 (55.6)	4 (44.4)	
Small cell carcinoma	14 (5.0)	7 (50.0)	7 (50.0)	
Squamous cell carcinoma	13 (4.7)	11 (84.6)	2 (15.4)	
Non – small cell carcinoma	6 (2.2)	6 (100)	0 (0)	0.10
Stage at diagnosis				
Local	61 (21.9)	35 (57.4)	26 (42.6)	
Regional/distant	180 (64.7)	112 (62.2)	68 (37.8)	
Unknown	37 (13.3)	23 (62.2)	14 (37.8)	0.50

*Where columns do not sum to the total, data were missing or unknown.

Table 2. Multivariable analysis of factors associated with ER-β positivity

Variable*	All subjects	Women	Men	Adenocarcinomas†	Matched subset‡
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Sex	0.54 (0.27-1.08)	—	—	0.40 (0.18-0.89)	0.42 (0.18-0.97)
Race	0.93 (0.50-1.73)	0.87 (0.42-1.80)	1.57 (0.39-6.31)	0.96 (0.49-1.87)	0.97 (0.36-2.64)
Age	1.01 (0.98-1.04)	1.01 (0.98-1.04)	1.04 (0.91-1.19)	1.01 (0.98-1.04)	1.04 (0.95-1.15)
Stage	1.29 (0.68-2.44)	1.21 (0.61-2.41)	2.80 (0.45-17.3)	1.24 (0.61-2.54)	1.98 (0.53-7.41)
Histology	1.05 (0.92-1.19)	1.12 (0.95-1.33)	0.91 (0.73-1.13)	2.35 (0.77-7.17)	1.01 (0.85-1.20)
Smoking	1.53 (0.58-4.08)	1.20 (0.38-3.72)	4.32 (0.56-33.7)	2.06 (0.73-5.85)	2.89 (0.55-15.3)
Pack-years	1.00 (0.99-1.01)	1.00 (0.99-1.02)	1.00 (0.98-1.03)	1.00 (0.99-1.01)	1.00 (0.98-1.02)
COPD	2.66 (0.83-8.51)	3.44 (0.90-13.1)	1.37 (0.11-17.3)	3.76 (1.00-14.1)	2.07 (0.21-20.7)

*Models compare males with females, Whites with other races, age in 1 year increments, regional and distant stage to local stage, histologies listed in Table 1, smoking yes/no, pack-years as a continuous variable, and COPD yes/no.
†Includes adenocarcinomas, bronchiolo-alveolar carcinomas, and adenosquamous carcinomas.
‡Subset of males and females matched on 5-year age group, histology, and stage.

in Table 3. After adjusting for sex, race, age at diagnosis, stage at diagnosis, smoking status, pack-years, histology, and history of tuberculosis, there was no difference in survival between subjects with ER-β–positive and ER-β–negative tumors for all subjects combined or in analyses restricted to case subsets (Table 3).

Analysis was repeated after stratifying by sex because there was a significant interaction between ER-β expression and sex ($P < 0.01$). Women with ER-β–positive tumors had a nonsignificant 69% increase in mortality compared with women with ER-β–negative tumors. Contrasting the effect in women, men with ER-β–positive tumors had a significant 55% (HR, 0.45) reduction in mortality after adjustment. This sex differential in survival associated with nuclear ER-β positivity is illustrated in Fig. 2. In men, ER-β–positive tumors were associated with better survival than ER-β–negative tumors ($P = 0.04$), whereas there was a nonsignificant trend in the opposite direction for women ($P = 0.13$).

This same pattern of survival was seen in analyses restricted to those with regional or distant-stage disease (Fig. 3). In men, ER-β–positive tumors were associated with a significant 55% (HR, 0.45) reduction in mortality ($P = 0.04$). There were too few subjects diagnosed at the local stage to calculate survival statistics. Similar findings were seen in analyses restricted to individuals with adenocarcinomas, in those without a previous cancer diagnosis, and in the matched subset. When the looser scoring criteria were used, the patterns were the same; however, in males, the findings were no longer statistically significant.

Survival analyses associated with ER-β in women by menopausal status were also conducted. In postmenopausal women, nuclear ER-β expression did not predict survival ($P = 0.81$). Premenopausal women with ER-β–negative tumors had better survival than premenopausal women with ER-β–positive tumors; however, this finding was based on very small numbers and was not statistically significant ($P = 0.09$). These analyses were adjusted for age, race, stage at diagnosis, smoking status, pack-years, tuberculosis history, histology, oral contraceptive use, and HRT use.

Discussion

This study showed the absence of nuclear ER-α in lung tissue and the presence of nuclear ER-β in normal lung, with increased presence in lung tumor tissue. In addition, nuclear ER-β expression varied by sex and was related differentially to survival in men, particularly for adenocarcinomas. Similar to

Table 3. HR values estimating risk of death associated with tumor ER-β positivity

Strata	All subjects*, HR (95% CI)	Women†, HR (95% CI)	Men‡, HR 95% CI
Total	1.01 (0.65-1.57)	1.69 (0.86-3.33)	0.45 (0.21-0.96)
Regional/distant stage	0.97 (0.62-1.51)	1.52 (0.76-3.02)	0.45 (0.21-0.96)
No previous cancer diagnosis	1.03 (0.63-1.67)	1.58 (0.87-2.88)	0.41 (0.18-0.97)
Matched subset§	0.84 (0.49-1.44)	1.46 (0.42-5.05)	0.45 (0.21-0.96)
Adenocarcinomas‖	0.93 (0.57-1.52)	1.56 (0.76-3.20)	0.32 (0.12-0.82)
Loose scoring criteria¶	1.23 (0.72-2.11)	1.39 (0.61-3.17)	0.72 (0.27-1.91)

*Adjusted for sex, race, age at diagnosis, stage at diagnosis, smoking status, pack-years, history of tuberculosis, and histology.
†Adjusted for race, age at diagnosis, stage at diagnosis, smoking status, pack-years, history of tuberculosis, histology, HRT use, menopausal status, and age at menarche.
‡Adjusted for race, age at diagnosis, stage at diagnosis, smoking status, pack-years, history of tuberculosis, and histology.
§Subset of males and females matched on 5-year age group, histology, and stage.
‖Includes adenocarcinomas, bronchiolo-alveolar carcinomas, and adenosquamous carcinomas.
¶More than 0% staining or cytoplasmic staining considered positive for ER-β.

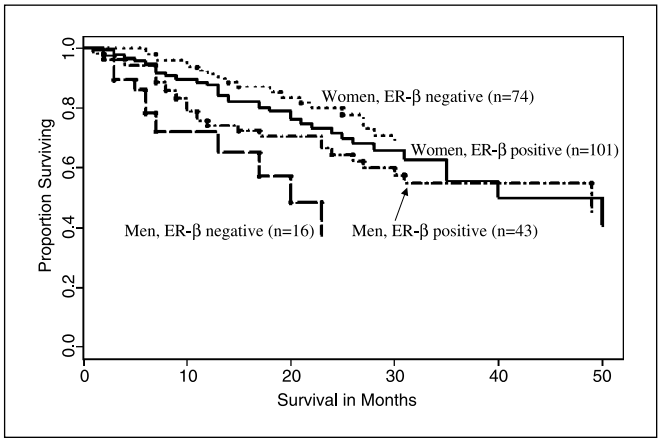

Fig. 2. Cox proportional hazard survival curves by sex and ER-β status.

the findings presented, other studies have failed to find ER-α expression in lung tumor tissue (15, 16). In contrast, Mollerup et al. (17) reported detectable ER-α mRNA levels in 31% of the tumor cell lines (n = 5) and in 82.6% (n = 38 of 46) of subjects. Further comparison to previous studies is limited because earlier studies were not able to distinguish ER-α from ER-β (14, 23–27). Truncated forms of ER-α were not identified in this study with the antibody used.

Sixty-one percent of lung cancer samples in this study stained positive for nuclear ER-β, which is consistent with previous studies. Omoto et al. (16) found 80% (n = 20 of 30) ER-β positivity using peroxidase-conjugated antichicken IgY rabbit antibody (Cosmobio, Tokyo, Japan). They also detected ER-β in normal tissue. Their hospital-based study included too few subjects to evaluate demographic or environmental characteristics in relation to ER status. Mollerup et al. (17) showed 100% ER-β mRNA expression in cell lines. Our study is the first large study to show sex differences in expression levels and evaluate the role of nuclear ER-β expression in survival. We evaluated our data using both conservative scoring criteria and loose scoring criteria, in adenocarcinomas only, in a matched subset of males and females, and after eliminating subjects with a previous cancer diagnosis with similar findings for each group.

Overall, women have been shown to have better survival after a lung cancer diagnosis than men (28). In our study, women also had better survival than men regardless of tumor ER-β expression. Among men, nuclear ER-β–positive tumors were associated with significantly better survival than negative tumors. In women, survival was not statistically significantly different by ER-β status, although there was the suggestion that survival was better in women with ER-β–negative tumors than for women with ER-β–positive tumors. There is only one other study that has evaluated lung tumor ER expression and survival. Canver et al. (14) found a significantly higher 3-year survival rate for women with ER–positive tumors than men; however, this study only followed 64 patients and was conducted before ER-β was identified so ER immunoreactivity cannot be definitively attributed to ER-β. In breast cancer, ER-β positivity has been shown to be associated with both better survival (29–31) and worse survival (32) in women being treated with tamoxifen. Both Davies et al. (33) and Palmieti et al. (34) suggest that the specific ER-β isoform is important, with ER-β2–negative breast tumors being associated with poorer survival. The antibody used in our study was raised against the COOH terminus of the ER-β1 isoform. Study of the role of additional isoforms is warranted. We also used a conservative scoring approach. Findings were in the same direction but not as strong, when including cytoplasmic staining only as positive. Mechanistic understanding of the role of ER-β in lung cancer will need to focus on the meaning of both nuclear and cytoplasmic staining.

Using Surveillance, Epidemiology, and End Results data, Moore et al. (35) reported that postmenopausal women with lung cancer (defined as age 51-70) had a 14% (95% CI, 1.03-1.27) higher risk of lung cancer–related deaths than premenopausal women (defined as ages 31-50) after adjusting for significant covariates. Although Moore et al. suggest that estrogen may confer a protective effect on outcome, they had no direct measures of menopausal status or tumor ER expression. In our study, no statistically significant differences in survival by menopausal status were detected; however, our study was too small to fully explore ER-β status by menopausal status. Additional studies with much larger sample sizes are needed.

Estrogens and ERs play important roles in regulating growth and differentiation of various tissues by acting through potentially two different ligand-activated mechanisms: one mechanism involves binding of a ligand to ER-α or ER-β nuclear receptors that induces a conformational change in the receptor, leading to an alteration of transcription by binding to the estrogen-response element or to transcription factors in the promoter regions of target genes (36, 37). Unlike ER-α, ER-β has been shown to decrease overall transcriptional activity (38–40) but can also function as a transcriptional activator at increased estradiol levels (39). Recent work using non–small cell carcinoma cell lines shows that estradiol promotes an association between ER-β and GRIP1/TIF2 coactivators that modifies gene expression (12) and stimulates cell growth (12, 41). When ER-β–expressing cell lines were treated with estradiol, E-cadherin and Id-2 levels increased, whereas antiestrogen treatment using ICI 182,780 resulted in decreased expression of these proteins. Variation in ER-β expression by sex in adenocarcinomas and the role of ER-β in predicting survival in men reported here suggests that future work should

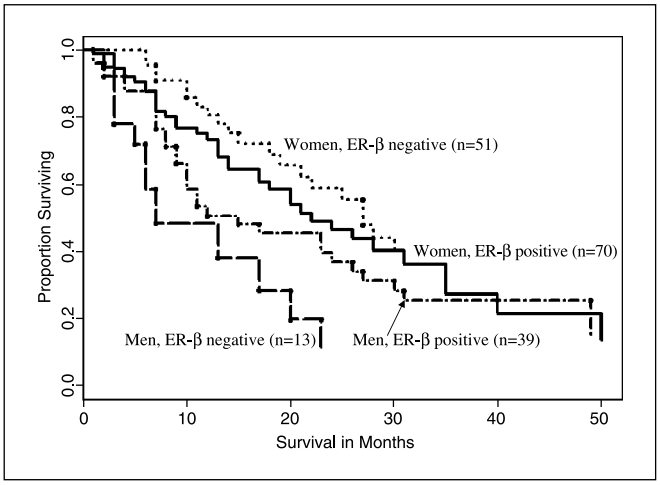

Fig. 3. Cox proportional hazard survival curves by sex and ER-β status among patients with regional or distant stage at diagnosis.

consider estradiol levels in conjunction with ER-β expression. Although not measured in this study, estrogen levels could vary by sex and in women by menopausal status. Local estrogen production may be of even greater significance in the lung, where aromatase and 17-β hydroxysteroid dehydrogenase are expressed (42, 43). Coombes et al. (44) recently report the development of fewer second primary lung cancers in breast cancer patients treated with exemestane, an aromatase inhibitor, compared with women treated with tamoxifen for 2 to 3 years, suggesting that aromatase may be associated with lung cancer risk.

A potential second mechanism involves cross-talk between ERs and growth factor receptor–mediated pathways in the plasma membrane (45). A recent study of cell growth of pulmonary lymphangioleiomyomatosis cells via ER-α and ER-β mechanisms by Yu et al. (46) found that estradiol and tamoxifen citrate stimulate both genomic events, through increased expression of c-*myc*, and nongenomic events, through rapid cytoplasmic activation of p44/42 mitogen-activated protein kinase. Functional interactions between ER-β and epidermal growth factor receptor have been shown as well (47). An epidermal growth factor receptor–dependent induction of phospho-p44/p42 mitogen-activated protein kinase was reported in response to estrogen in non–small cell carcinoma cell lines expressing ER-β. Stabile et al. (47) also report that epidermal growth factor receptor protein is down-regulated in response to estrogen and up-regulated by antiestrogens in these same cell lines. Their work provides support for lung cancer treatment using a combination of fulvestrant, an estrogen antagonist, and gefitinib, a selective epidermal growth factor receptor tyrosine kinase inhibitor.

The study presented is descriptive and provides important information about nuclear ER-β expression in lung cancer. Although extensive antibody testing was done and slides were interpreted by a single pathologist, the possibility remains that misclassification of ER-β status and histology may have occurred in some cases. Our negative ER-α results are consistent with the belief that ER-α protein is either expressed at low levels in the lung or not at all (48, 49); however, fragmented ER-α may have escaped detection despite the use of two separate antibody assays. Extensive testing and evaluation of antibodies for ER-β assays add strength to our findings. The findings represented here include the largest number of samples in any study to date to evaluate nuclear ER-β expression in the lung and provide the first examination of potential synergistic effects between environmental risk factors, subject characteristics, ER status, and survival. However, even with our large study, some findings are based on small sample sizes. Not all lung cancer patients have surgery and in our study sufficient tumor tissue was available for only 32.5% of those interviewed. Fewer samples were available for men than for women. Another potential limitation in our study was the differential age distribution for men and women; however, findings did not change when analyses were repeated using an age-, histology-, and stage-matched subset. Approximately 85% of the lung cancers included were adenocarcinomas and although ER-β expression did not differ by histologic type, results most specifically apply to adenocarcinomas. Larger studies of histologic types other than adenocarcinomas should be conducted. Survival analyses used all causes of death, not lung cancer-specific deaths; however, most lung cancer patients die from their disease.

In summary, we have identified differential nuclear ER-β expression in normal and lung tumor tissue, more frequent nuclear ER-β expression in men than in women particularly for adenocarcinomas, and survival differences in men by ER-β status. Nuclear ER-β expression was extremely common in these lung cancers opening up an avenue for translational research. Identifying ER-β status of a tumor may hold clinical value as a prognostic factor for predicting response to estrogen antagonists. Definitive conclusions pertaining to physiologic and tumorigenic consequences of ER-β expression in human lung await additional studies. Inclusion of risk factor data is important in this work because level and composition of circulating estrogens can be affected by smoking, age, race, oral contraceptive use, and HRT use (7, 50) and may, therefore, be indirectly related to ER-β function through an estrogen-mediated mechanism. Our studies are ongoing and further analyses will include testing for associations between ER status and genetic polymorphisms associated with estrogen metabolism, a more detailed evaluation of HRT use and menopausal status, ER-β polymorphisms, and alternative ER-β isoform expression.

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