Aromatase Inhibitors in Human Lung Cancer Therapy

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Abstract

Lung cancer is the most common cancer in the world. It is a highly lethal disease in women and men, and new treatments are urgently needed. Previous studies implicated a role of estrogens and estrogen receptors in lung cancer progression, and this steroidal growth-stimulatory pathway may be promoted by tumor expression and activity of aromatase, an estrogen synthase. We found expression of aromatase transcripts and protein in human non–small cell lung cancer (NSCLC) cells using reverse transcription-PCR and Western immunoblots, respectively. Aromatase staining by immunohistochemistry was detected in 86% of archival NSCLC tumor specimens from the clinic. Further, biological activity of aromatase was determined in NSCLC tumors using radiolabeled substrate assays as well as measure of estradiol product using ELISA. Significant activity of aromatase occurred in human NSCLC tumors, with enhanced levels in tumor cells compared with that in nearby normal cells. Lung tumor aromatase activity was inhibited by anastrozole, an aromatase inhibitor, and treatment of tumor cells *in vitro* with anastrozole led to significant suppression of tumor cell growth. Similarly, among ovariectomized nude mice with A549 lung tumor xenografts, administration of anastrozole by p.o. gavage for 21 days elicited pronounced inhibition of tumor growth *in vivo*. These findings show that aromatase is present and biologically active in human NSCLCs and that tumor growth can be down-regulated by specific inhibition of aromatase. This work may lead to development of new treatment options for patients afflicted with NSCLC. (Cancer Res 2005; 65(24): 11287-91)

Introduction

Lung cancer is the leading cause of death from cancer in both women and men in the United States. In view of the recent surge in female death rates from lung cancer (1), the potential role of both exogenous and endogenous estrogens in lung cancer development, especially adenocarcinoma in women, requires further investigation (1–3). Estrogens contribute to differentiation and maturation in normal lung (4) and also stimulate growth and progression of lung tumors (3, 5). These biological effects are mediated by estrogen receptors (ER), with ER transcripts and proteins reported in most non–small cell lung cancers (NSCLC; refs. 3, 5–10). Moreover, as in breast, aromatase, a cytochrome P450 enzyme complex, mediates synthesis of estrogens in lung tissues (5, 11), and local production of estrogens in women and in men could affect lung tumor progression in ER-expressing malignancies (5, 12). Potent third-generation aromatase inhibitors, such as anastrozole, interact competitively with heme groups of cytochrome P450 components of aromatase. As in breast cancer (12), aromatase inhibitors may prove useful to block estrogen synthesis and estrogen-induced growth in lung cancers. Thus, we investigated the role of aromatase in lung cancer by assessing expression and biological activity of the estrogen synthase in lung cancers and we confirmed tumor expression of ER. In addition, antitumor efficacy of anastrozole was tested in experiments with human lung tumors growing *in vitro* and as xenografts in nude mice.

Materials and Methods

Cell lines and reagents. NSCLC cells H23, A549, A427, A125, H2122, H1299, H157, H647, H460, H1650, H3255, and H226, and control breast cancer cells SKBR3, MCF-7, and T47D were from the American Type Culture Collection (Manassas, VA). Cells were maintained as before (5, 13). Anastrozole was from AstraZeneca (Wilmington, DE) and TeamLife Research (New York, NY), whereas testosterone and androstenedione were from Steraloids (Newport, Rhode Island) and Casodex (bicalutamide) was from AstraZeneca.

Antibodies and Western blotting. Antiaromatase antibody (CYP19) C-16 was from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies to ERα (1D5) and ERβ (D7N) were from Invitrogen/Zymed (Carlsbad, CA). Cells were prepared for electrophoresis and Western blotting as before (5, 13).

RNA isolation and reverse transcription-PCR assay of aromatase. Total RNA was extracted from cells using TRIzol reagent (Invitrogen) as before (14). In brief, 2 μg total RNA were reverse transcribed to first-strand cDNA with SuperScript II reverse transcriptase (Invitrogen). PCR was done with Platinum Taq polymerase using the "Hot Start" method (95°C/3 minutes) followed by 40 cycles of 94°C/15 seconds, annealing of 60°C/30 seconds (for aromatase), and 50°C/30 seconds [for glyceraldehyde-3-phosphate dehydrogenase (GAPDH)], 68°C/30 seconds, followed by a final extension of 68°C for 10 minutes.

Aromatase activity in cell lines and archival tumor specimens. Aromatase activity was assessed by use of radiolabeled substrate, [1β-^{3}H]androst-4-ene-3,17-dione (Perkin-Elmer, Boston, MA) with established methods in lung cells (16) as well as in previously frozen lung NSCLC specimens (17). Controls included use of cells or tissues without [1β-^{3}H]androst-4-ene-3,17-dione and deletion of cells or tissues.

As aromatase catalyzes conversion of androstenedione and testosterone to estrone and estradiol, respectively, its activity was determined by production of estradiol after treatment with testosterone. Cells were plated in RPMI medium with 10% fetal bovine serum (FBS). After reaching 50% confluence, cells were rinsed with PBS and incubated with phenol red-free medium with 1% dextran-coated, charcoal-treated FBS for 48 hours to deplete estrogen (5, 13). Thereafter, medium was refreshed and cells were treated with testosterone for 48 hours. Estradiol production was measured with ELISA (ALPCO Diagnostics, Windham, NH) using the instructions of the manufacturer in four independent experiments.

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Immunohistochemical assays. Formalin-fixed, paraffin-embedded lung tumor samples from 53 patients were obtained, and immunohistochemistry was done by established methods (5). Negative controls (deletion of primary antisera as well as IgG isotype control) and positive controls (tissues with known aromatase) were prepared in parallel.

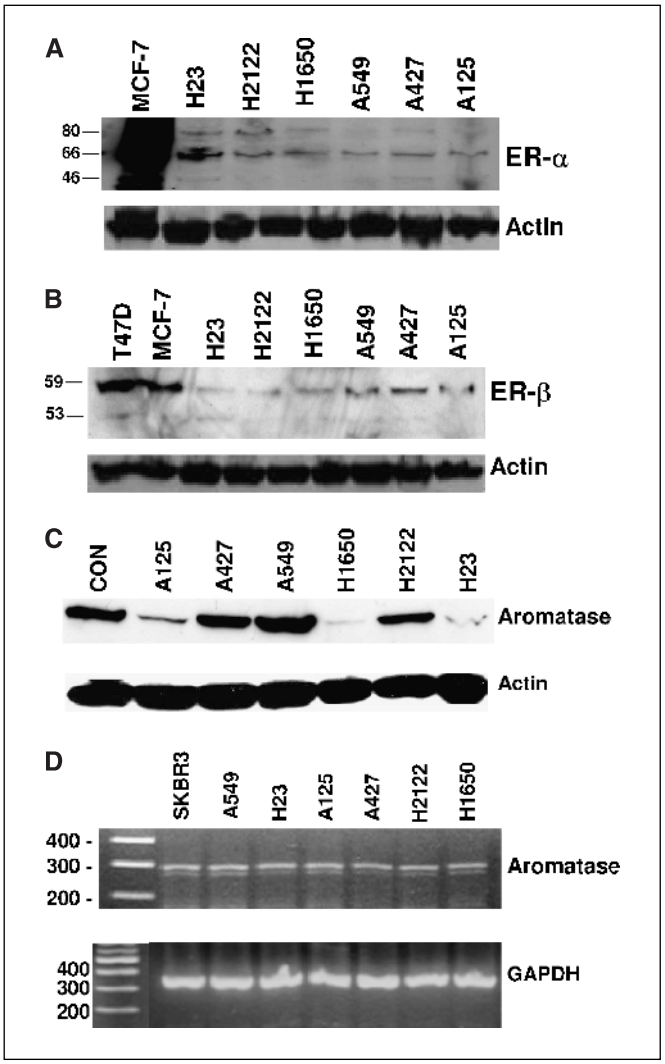


Figure 1. Estrogen receptors and aromatase are expressed in human lung cancer cells. *A,* using gel electrophoresis with Western blot, ERα expression is shown in six representative lung NSCLC cell lines. *MCF-7,* positive control, breast cancer cell line MCF-7 with known high expression of ERα. *H23, H2122, H1650, A549, A427*, and *A125,* lung cancer cell lines as noted. Most samples are from male donors, except for H2122. *Bottom,* actin loading controls. *B,* using electrophoresis with Western blot, expression of ERβ is shown in most NSCLC cell lines. In addition, breast cancer cell line T47D also contains abundant ERβ. *Bottom,* actin loading controls. *C,* using electrophoresis and Western blot, aromatase expression in six representative lung cancer cell lines is shown. The first lane is a positive control breast cancer cell line SKBR3 with known high levels of aromatase (*CON*). The remaining lanes contain the specified lung NSCLC cells. *Top,* aromatase; *bottom,* actin loading control. *D,* RT-PCR amplification for assay of aromatase mRNA from NSCLC cells. Total RNA was isolated from NSCLC cells as noted and the breast cancer cell line SKBR3, with cDNA amplification and PCR done as before (14). PCR amplification of cDNA was done using the following primers: ARO-3 (forward): 5′-GAATATTGGAAGGATGCACAGACT-3′ and ARO-4 (reverse): 5′-GGGTAAAGATCATTTCCAGCA TGT-3′ resulting in a 294 bp product (15). For control of RNA integrity and relative abundance normalization, GAPDH was amplified (14) with an expected 355 bp product. Amplicons resolved on agarose gels were visualized by ethidium bromide staining. The size of the expected aromatase fragment (aromatase) is 294 bp. Corresponding analyses of GAPDH (364 bp fragment) confirmed integrity of the RNA samples. Note that the same representative lung cancer cell lines were used for RT-PCR and for Western immunoblots, whereas different breast cancer controls were used to determine ER or aromatase expression.

Cell proliferation assays. Lung tumor cells were plated in RPMI medium with 10% FBS. After cells reached 50% confluence, they were rinsed with PBS and incubated with phenol red–free medium with 1% dextran-coated, charcoal-treated FBS for 48 hours (13). Then, the medium was refreshed and cells were treated with anastrozole. In addition, androstenedione or estrogen was added to some cells without drugs. After 72 hours, cells were counted manually (13).

Inhibition of aromatase in cell lines. A549 cells were plated and grown to 50% confluence (13); cells were then washed and treated with anastrozole for 48 hours. Aromatase activity was assessed in triplicate (16), with untreated A549 cells used as controls.

***In vivo* tumor xenograft studies.** Ovariectomized nude mice at 6 weeks of age were from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed in a pathogen-free environment with controlled light and humidity and received food and water *ad libitum*. To prepare lung tumor xenografts, A549 cells (2×10^8 per mouse) were injected s.c. with 1 tumor/mouse (13). Androstenedione (0.1 mg/mouse) was injected s.c. daily in each mouse throughout the experiment to provide substrate for aromatase. After tumors reached 50 to 75 mm^3, mice were randomized into uniform groups based on animal weight and tumor volume for treatment with control or anastrozole (13, 18). Anastrozole was administered by daily p.o. gavage at 0.1 mg/kg (20 μg/mouse) in water. Treatment duration was 21 days. Tumor volumes were measured every 3 to 4 days, with tumor volume calculated by ($l \times w \times h$), where l is tumor length, w is tumor width, and h is tumor height in mm.

Statistical analyses. Data were analyzed using ANOVA and Student's t tests (18). Tests of statistical significance were two-sided, with differences significant if $P < 0.05$.

Clinical samples. Patient specimens and information were collected under Institutional Review Board–approved and Health Insurance Portability and Accountability Act–compliant protocols at University of California at Los Angeles Medical Center.

Results

Aromatase and estrogen receptor expression in non–small cell lung cancer cell lines. Expression of ERα and ERβ proteins in lung tumor cells was assessed by Western immunoblotting, with results for six representative lung cell lines and for selected breast cancer controls shown in Fig. 1*A*. The classic 67 kDa ERα, as well as a 46 kDa variant, occurs in MCF-7 breast cells. The several lung cancer cells also express these ERα forms, albeit at lower levels than breast cells. Lung cells also contain a larger 80 kDa ERα form that may represent another variant with an in-frame duplication of exons 6 and 7 (2). As shown in Fig. 1*B*, ERβ forms at predominantly 59 kDa were found in lung cancer cells, as well as in breast cells (2, 5).

Expression of aromatase protein in lung tumor cells was assessed, with results for six representative cell lines shown (Fig. 1*C*). As in SKBR3 breast cancer cells with known high aromatase expression, the enzyme occurred in most lung cancer cells, with molecular size ~54 kDa (12). To confirm these findings, reverse transcription-PCR (RT-PCR) was used to detect aromatase transcripts in lung cells. As shown in Fig. 1*D*, a 294 bp transcript was detected in all lung cancer cells examined, consistent with earlier reports (15). A similar RT-PCR product was detected in SKBR3 cells with high aromatase levels.

Aromatase activity in human lung tumors. To assay aromatase activity, we measured conversion of androstenedione substrate to estrogens (16). Activity occurred in all lung cancer cell lines tested and ranged from 0.37 to 3.21 pmol/mg/h, with average aromatase activity of 1.49 ± 0.23 pmol/mg/h (see Fig. 2*A*). As

expected, SKBR3 cells had high activity, 5.84 pmol/mg/h, a level exceeding that of most breast cells. In our study, MCF-7 breast cells had <5% of SKBR3 cell activity (not shown), as in previous reports (16). Among a total of 12 human NSCLC cell lines evaluated, aromatase activity was highest in H2122, A549, and H647 (not shown) cells and lowest in H23 cells (Fig. 2*A*).

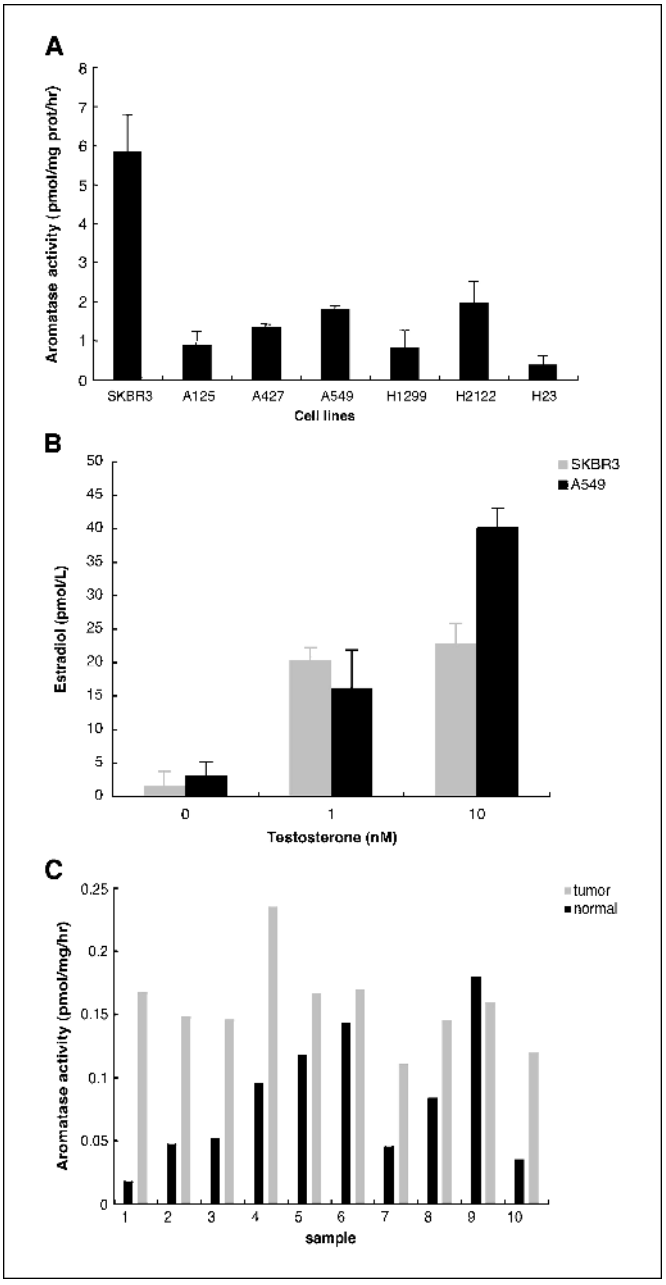
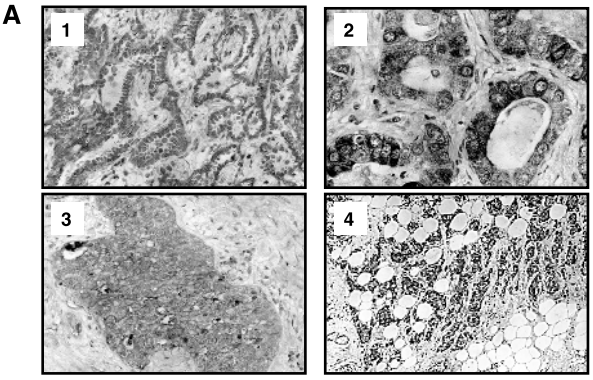


Figure 2. Aromatase activity occurs in human NSCLCs. *A,* radioassay of aromatase activity in lung NSCLC cell lines. Levels of substrate metabolism in lung tumor cell lines (*abscissa*) are compared with that in SKBR3 breast cells. See text for details. *B,* aromatase activity in A549 and SKBR3 cells was measured by production of estradiol following treatment with testosterone. The greatest increment in the production of estradiol occurred after 48 hours treatment with 10 nmol/L testosterone in A549 lung cancer cells. Estradiol standards were provided by the manufacturer, and negative controls included cells grown but not treated with testosterone. *C,* radioassay of aromatase activity in previously frozen archival lung tumor specimens from the clinic, with normal tissue (*black columns*) compared with tumor tissue (*gray columns*) from the same donors. Lung tumors examined were obtained from six male and four female patients, with tumor histologies including seven adenocarcinomas, two squamous carcinomas, and one adenosquamous carcinoma. The highest tumor aromatase activity was in a squamous carcinoma from a male patient (*sample 4*), whereas the lowest was in an adenocarcinoma from a male patient (*sample 7*).



B

	Female positive	Female total	Male positive	Male total	Total Positive Tumors (%)
Adenocarcinoma	25	28	11	12	88
Squamous	3	3	4	5	87
Bronchioalveolar	0	1	3	3	75
Adenosquamous	1	1	0	0	100
Total Tumors	29	33	18	20	86

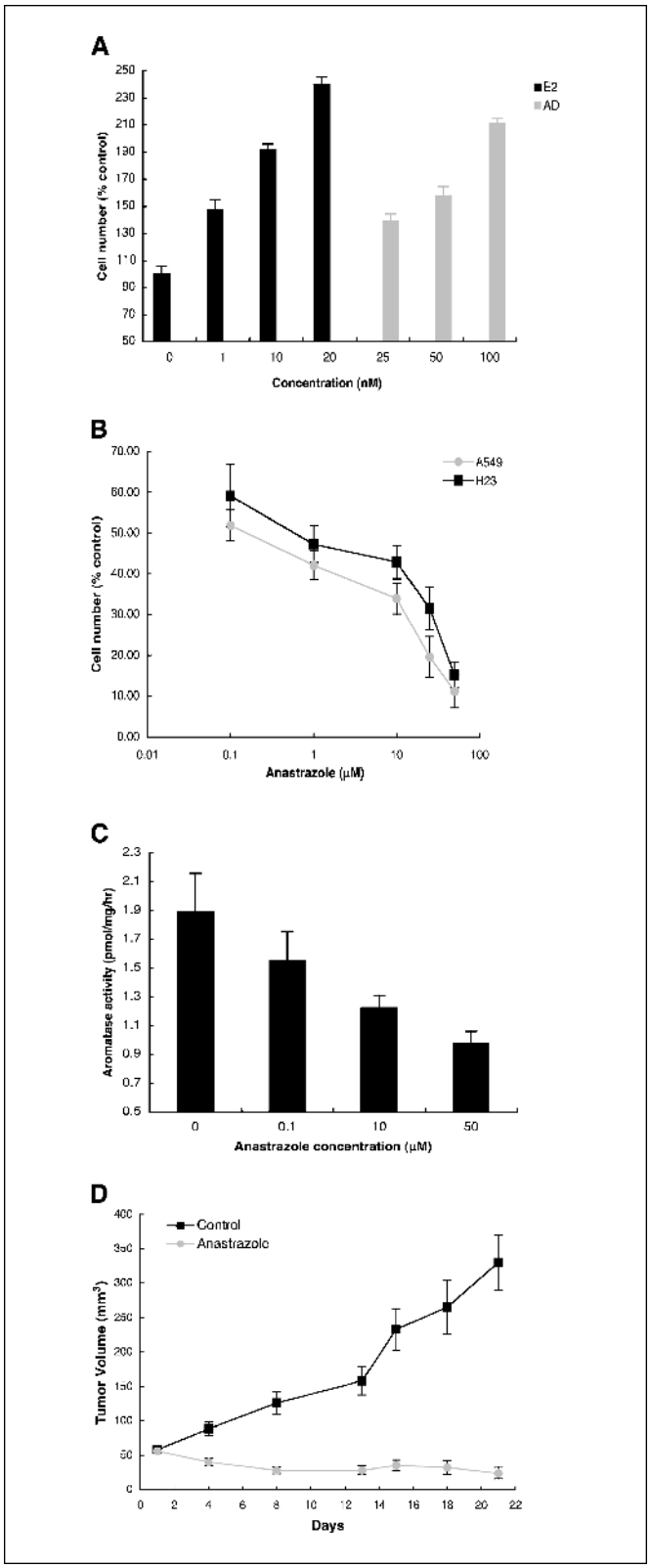
Figure 3. Aromatase expression by immunohistochemistry is present in most archival lung NSCLC tumor specimens. *A,* representative examples of positive aromatase staining (>15% of cells with specific staining) in different tumor specimens, including bronchioalveolar carcinoma (*1*); adenocarcinoma (*2*); squamous carcinoma (*3*); and breast cancer control (*4*). Specific staining was verified by established methods, including exclusion of primary antibody, use of a specific blocking peptide (Santa Cruz Biotechnology) and use of negative control cells, MCF-7, with negligible aromatase (5). Aromatase staining localized predominantly in tumor epithelial cells, with less staining in surrounding stromal tissues; specific staining was never or rarely observed in lymphocytes or interstitial surrounding tissue, respectively. *B,* summary of aromatase expression by immunohistochemistry in 53 human tumor samples. Using a cutoff score of 15% total cell staining for aromatase positivity, 86% of lung NSCLC specimens overall expressed aromatase, with 88% of female and 86% of male samples positive (see display and text for details).

Aromatase activity was also quantitated by estradiol production after treatment of A549 cells with testosterone at 1 to 10 nmol/L (Fig. 2*B*). Increased testosterone levels promoted a significant increase in estradiol biosynthesis in NSCLC cells, providing further evidence for functional aromatase ($P < 0.001$).

To assess aromatase activity in clinical material, archival lung tumor specimens were tested by radioassays with [1β-^{3}H]androst-4-ene-3,17-dione substrate (17). Paired tumor and neighboring normal tissue was examined from 10 donors (Fig. 2*C*). Aromatase activity in normal tissue averaged 0.081 ± 0.052 pmol/mg/h, whereas corresponding tumors had significantly higher activity, averaging 0.15 ± 0.03 pmol/mg/h ($P = 0.001$; Fig. 2*C*).

Immunohistochemical assay for aromatase in lung tumors. To determine the prevalence of aromatase in human NSCLC, we assessed 53 archival tumor specimens by immunohistochemistry. Representative examples of specific staining patterns of different tumor types classified as aromatase-positive, including a positive control breast cancer specimen, are shown in Fig. 3*A*. Staining was primarily in tumor epithelial cells, with distinct clusters of positive-staining tumor cells throughout (Fig. 3*A*). Often, adjacent normal epithelium of bronchioles also exhibited weak aromatase staining,

but supporting stromal tissues had little aromatase. Macrophages contained aromatase in some samples, about twice as often in females as in males. Aromatase staining was not observed in lymphocytes or interstitial surrounding tissue. Using a cutoff score of 15% total cell staining for aromatase positivity, tumor specimens were classified as negative (≤15% stained) or positive (>15% stained), with examples of aromatase-positive lung tumors in Fig. 3*A*. Using this evaluation system, 86% of lung tumor specimens expressed aromatase (see summary; Fig. 3*B*). This finding was relatively uniform among different lung NSCLC histologies evaluated, with 88% of adenocarcinoma and 87% of squamous carcinomas positive for aromatase. Of the limited number of bronchioalveolar and adenosquamous samples available, 75% and 100%, respectively, scored positive (Fig. 3*B*). Using these scoring criteria, 88% of female and 86% of male NSCLC samples were positive.



Aromatase inhibitor blocks lung tumor growth *in vitro* and decreases aromatase activity. As reported previously, both A549 and H23 lung tumor cells express ER (2, 5, 9). To assess estrogen dependence of lung cells for growth, cell proliferation in response to estrogen and androstenedione, an aromatase substrate to promote cellular estrogen production, was tested. As shown in Fig. 4*A*, increasing doses of estrogen from 1 to 20 nmol/L elicit enhanced proliferation of A549 cells. At 20 nmol/L, estrogen induced a significant 2.4-fold increase in cell proliferation ($P < 0.001$) as before (2, 5). Similarly, androstenedione at increasing doses significantly stimulated proliferation of A549 cells (Fig. 4*A*). At 100 nmol/L, androstenedione elicited a 2.1-fold increase in A549 cell growth ($P < 0.001$).

To assess direct effects of an aromatase inhibitor on lung cancer cell proliferation, tumor cells were treated with or without anastrozole *in vitro*. Anastrozole significantly reduced cell growth in a dose-dependent manner ($P < 0.001$; Fig. 4*B*). Fifty percent growth inhibition occurred at 0.1 mmol/L anastrozole for A549 and at 3.5 mmol/L anastrazole for H23 cells, suggesting that tumors with both low and relatively higher levels of aromatase are sensitive to aromatase inhibition (Fig. 4*B*).

The ability of anastrozole to suppress aromatase activity in lung cells was also tested (Fig. 4*C*). A549 cells were treated 48 hours with anastrozole and aromatase activity was then determined and compared with controls. A significant dose-dependent decrease in

Figure 4. Aromatase inhibitor, anastrozole, blocks growth of lung tumor cells *in vitro* and human lung tumor xenografts in nude mice *in vivo*. *A,* effect of estrogen (*E2*) and androstenedione (*AD*) on promotion of growth in A549 human lung cancer cells. Cells were serum deprived and treated for 48 hours with estrogen or androstenedione. Treatment with both agents led to stimulation of cell growth. *B,* anastrozole reduces growth of A549 and H23 NSCLC cells *in vitro*. Both cell lines were serum deprived for 48 hours and treated with anastrozole for 72 hours at concentrations ranging from 100 nmol/L to 25 μmol/L. Of note, growth of A549 cells in the absence of estradiol with steroid-depleted medium, a condition that mimics anastrozole treatment, suppresses cell proliferation compared with controls ($n = 3$; $P < 0.001$); bicalutamide (5-50 μmol/L), a competitive antagonist of androgen action, does not alter the antiproliferative effect of anastrozole in A549 cells ($n = 2$; data not shown). *C,* anastrozole inhibits aromatase activity in A549 cells. A549 cells were grown to 50% confluence and treated with anastrozole for 48 hours at 0.1, 10, 25, or 50 μmol/L. Aromatase activity was then measured using radioassays as described in the text. *D,* aromatase inhibitor suppresses growth of human lung tumor xenografts *in vivo*. A549 lung tumor cells were implanted as s.c. xenografts in ovariectomized nude mice. Mice were supplemented with androstenedione, an aromatase substrate, s.c. each day throughout the experiment. When tumors achieved a limiting size of 50 to 75 mm^3, mice were randomized into two treatment groups ($n = 5$): control vehicle or anastrozole (20 μg/mouse/d given by p.o. gavage). Groups were balanced according to animal weight and tumor volumes to achieve similar tumor volume averages and animal weights in each group at the start of the experiment (13, 18). Treatments with anastrozole were continued for 21 days. Significant inhibition of tumor growth was found in the anastrozole group compared with control ($P < 0.001$). *Points,* mean for tumor volumes in each group; *bars,* SE.

aromatase activity was found, with more pronounced aromatase inhibition at 10 to 50 μmol/L anastrozole ($P \leq 0.01$). This dose range corresponds well with concentrations noted to be more effective in growth inhibition *in vitro* (Fig. 4*B*).

Aromatase inhibitor suppresses growth of lung tumor xenografts *in vivo*. To assess *in vivo* antitumor effects of anastrozole, A549 cells that express aromatase and ERβ (2, 5, 9) and respond well to estrogen (2) were grown as xenografts in ovariectomized nude mice treated daily with aromatase substrate, androstenedione. After tumors grew to a limiting size of 50 to 75 mm^3, mice were divided into two treatment groups, control vehicle and anastrozole, and then treated for 21 days. Results in Fig. 4*D* show that, by day 21, there was exponential growth of tumors in control mice, whereas growth of tumors in mice treated with anastrozole was significantly suppressed by >90% compared with controls ($P < 0.001$).

Discussion

The current data and previous findings suggest that treatments targeted to estrogen signaling pathways in lung cancer may have previously unsuspected antitumor efficacy (2, 5, 9, 10, 19). In nonreproductive tissues, the lung possesses among the highest levels of ER transcripts, especially those for ERβ (7). Our results confirm that wild-type and variant forms of ERα and ERβ occur in lung tumors (2, 5, 8, 9, 19) and participate in growth promotion in lung cancer (2, 5, 19). Moreover, we show with different methods that local production of estrogens occurs in lung tumor cells and NSCLC tissue samples through the action of aromatase, and ligands generated may then bind and activate ER. Aromatase activity occurs in most tumors from male and female patients with different NSCLC histologic types. The majority of lung tumors in this study were adenocarcinomas, the most common histologic type of lung cancer in young persons, women of all ages, and never smokers of both sexes (1). Further, levels of aromatase activity were significantly greater in tumors compared with those in nearby normal tissue. Similarly, among archival lung NSCLC evaluated by immunohistochemistry, aromatase localized predominantly in tumor epithelial cells, with less staining in surrounding stromal tissues. Of note, aromatase also occurred in normal epithelial cells distant from primary tumor and in some infiltrating macrophages. In breast, macrophages constitute another source of estradiol to promote cell proliferation (20).

To further assess the notion that estrogen signaling promotes lung cancer progression, antitumor activity of aromatase inhibitors was assessed. Anastrozole, when administered daily to postmenopausal women with breast cancer, inhibits *in vivo* aromatization by 96.7% and clinical trials show strong antitumor efficacy of anastrozole in breast cancer (12, 16). Similarly, in our study, lung aromatase activity was significantly reduced by anastrozole, and treatment of lung tumor cells *in vitro* and xenografts *in vivo* with anastrozole elicited a corresponding suppression of tumor growth compared with controls. Therapeutic targeting of lung cancer to block specific receptor pathways, such as estrogen signaling, may provide new options for patients afflicted with lung malignancies.

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References

1. Patel JD, Bach PB, Kris MG. Lung cancer in US women: a contemporary epidemic. JAMA 2004;291:1763–8.
2. Taioli E, Wynder EL. Endocrine factors and adenocarcinoma of the lung in women. J Natl Cancer Inst 1994;86:869–70.
3. Stabile LP, Davis ALG, Gubish CT, et al. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor α and β and show biological responses to estrogen. Cancer Res 2002;62:2141–50.
4. Patrone C, Cassel T, Pettersson K, et al. Regulation of postnatal lung development and homeostasis by estrogen receptor β. Mol Cell Biol 2003;23:8542–52.
5. Pietras RJ, Marquez DC, Chen H-W, Tsai E, Weinberg O, Fishbein M. Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells. Steroids 2005;70:372–81.
6. Beattie CW, Hansen NW, Thomas PA. Steroids receptors in human lung cancer. Cancer Res 1985;45:4206–14.
7. Couse JF, Lindzey J, Grandien K, Gustafsson JA, Korach KS. Tissue distribution and quantitative analysis of estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) messenger ribonucleic acid in the wild-type and ERα-knockout mouse. Endocrinology 1997;138:4613–21.
8. Kirsch EA, Yuhanna IS, Chen Z, German Z, Sherman TS, Shaul PW. Estrogen acutely stimulates endothelial nitric oxide synthase in H441 human airway epithelial cells. Am J Respir Cell Mol Biol 1999;20:658–66.
9. Mollerup S, Jorgensen K, Berge G, Haugen A. Expression of estrogen receptor α and β in human lung tissue and cell lines. Lung cancer 2002;37:153–9.
10. Kawai H, Ishii A, Washiya K, et al. Estrogen receptor α and β are prognostic factors in non-small cell lung cancer. Clin Cancer Res 2005;11:5084–9.
11. Pezzi V, Mathis JM, Rainey WE, Carr BR. Profiling transcript levels for steroidogenic enzymes in fetal tissues. J Steroid Biochem Mol Biol 2003;87:181–9.
12. Brodie A, Long B, Lu Q. Aromatase expression in the human breast. Breast Cancer Res Treat 1998;49:S85–91.
13. Pietras RJ, Arboleda J, Reese D, et al. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene 1995;10:2435–46.
14. Garban HJ, Bonavida B. Nitric oxide disrupts H_2O_2-dependent activation of nuclear factor κB. Role in sensitization of human tumor cells to tumor necrosis factor-α-induced cytotoxicity. J Biol Chem 2001;276:8918–23.
15. Long BJ, Tilghman SL, Yue W, Thiantanawat A, Grigoryev DN, Brodie AM. The steroidal antiestrogen ICI 182,780 is an inhibitor of cellular aromatase activity. J Steroid Biochem Mol Biol 1998;67:293–304.
16. Kinoshita Y, Chen S. Induction of aromatase (CYP19) expression in breast cancer cells through a nongenomic action of estrogen receptor α. Cancer Res 2003;63:3546–55.
17. Lu Q, Nakmura J, Savinov A, et al. Expression of aromatase protein and messenger ribonucleic acid in tumor epithelial cells and evidence of functional significance of locally produced estrogen in human breast cancers. Endocrinology 1996;137:3061–8.
18. Pegram MD, Finn RS, Arzoo K, Beryt M, Pietras RJ, Slamon DJ. The effect of HER-2/neu overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. Oncogene 1997;15:537–47.
19. Stabile LP, Lyker JS, Gubish CT, Zhang W, Grandis JR, Siegfried JM. Combined targeting of estrogen receptor and epidermal growth factor receptor in non-small cell lung cancer shows enhanced antiproliferative effects. Cancer Res 2005;65:1459–70.
20. Mor G, Yue W, Santen RJ, et al. Macrophages, estrogen and the microenvironment of breast cancer. J Steroid Biochem Mol Biol 1998;67:403–11.

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