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Expression of aromatase CYP19 and its relationship with parameters in NSCLC

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1. ABSTRACT

Human aromatase (CYP19) responsible for the conversion of androgens to estrogens is expressed not only in gonads and adrenals but also in many other tissues, including normal lungs and lung cancers. To investigate the involvement of CYP19 in lung cancer development, purified CYP19 protein and antibody are required. In this study, we have developed an efficient expression method of human aromatase in *E. coli (*>1000 nmol/L culture). The protein purified from *E. coli* was used to raise an antibody against the human CYP19 in rabbits. The resulting antibody showed a high titer judged by ELISA, which allowed us to determine the expression of CYP19 in non-small cell lung cancer (NSCLC). Of 78 NSCLC specimens from Japanese patients, 50 (64%) NSCLC aberrantly expressed CYP19. This CYP19 expression in NSCLC was independent of any clinical and pathological parameters as well as the expression of other P450s, except tumor stage. The results suggest that the aromatase inhibitors might be useful for the management of non-small cell lung cancer in postmenopausal women.

2. INTRODUCTION

Estrogens are involved in the differentiation and maturation of normal lungs in the fetus as well as in the postnatal lung development (1). In both male and female rhesus monkey, lung tissues express aromatase (CYP19), along with steroid sulfatase, 3β-hydroxysteroid dehydrogenase, and 17β-hydroxysteroid dehydrogenase, suggesting that the lung in primates may produce estradiol from dehydroepiandrosterone sulfate (2). Normal human lungs express both estrogen receptor α (ERα) and β (ERβ), suggesting that estrogen signaling plays a biological role in the lung (3). Based on results from the investigation of ERα and ERβ knockout mice and ovariectomy of adult mice, estrogens are required for the maintenance of alveoli, at least, in mice (4).

In addition to the importance of estrogen signaling in the normal lung, estrogens also stimulate the growth and progression of lung tumors (5) through the action of estrogen receptors (ER) (6). The number of female death from pulmonary adenocarcinoma is increasing (7), which

might be associated with estrogen function in the lung. Taioli, *et al.* showed that early age at menopause, before or at the age of 40 years, was significantly associated with a decreased risk for adenocarcinoma (OR; odds ratio = 0.3) and that the use of estrogen replacement therapy (ERT) was associated with the incidence of adenocarcinoma (OR = 1.7), suggesting that exogenous and endogenous estrogens play a role in the etiology and development of lung cancer, especially adenocarcinoma in women (8-10).

Upon the immunohistochemical study of ER and progesterone receptor (PgR) in non-small-cell lung cancer (NSCLC), Su *et al.* showed that the 38.8% (19/49) NSCLC had either an ER or PgR positive status, of which one was ER+/PgR+, 2 were ER+/PgR-, and 16 were ER-/PgR+ although there was no statistical difference in hormone receptors status with sex and histological subtypes (11). From the investigation of mRNA expression of ERα and ERβ in cultured human NSCLC cells, Stabile, *et al.* also concluded that estrogens could potentially promote lung cancer, either through direct actions on preneoplastic or neoplastic cells or through indirect actions on lung fibroblasts (3), which suggests that anti-estrogens might have therapeutic value to treat or prevent lung cancer.

Although estrogens are synthesized by aromatase (CYP19), mostly in gonads and adrenals, CYP19 is also expressed in extragonadal tissues, including brain, skin, adipose and lung tissues (12, 13). In the investigation of the CYP19 expression in lung cancer (n = 53), CYP19 was detected in 86% of NSCLC, and the CYP19 enzyme expressed in the tumors was biologically active (13). Therefore, the local expression and activities of CYP19 in NSCLC may play a promotive role in the estrogen-dependent growth-stimulation of the tumors. Lung cancer is the number one cause of death from cancer in both men and women in Japan as it is in the United States. Although lung cancer affects men more than women, the gap between men and women is less in the United States compared with in Japan. Therefore, the ratio of estrogen-dependent lung cancer might be different between the two countries or among countries, which led us to investigate the relationship of aromatase expression in lung cancer with genders in Japanese patients. In spite of increasing importance for detailed analysis of aromatase expression in tissues, availability of good antibodies against human aromatase is limited. In this study, we developed the *E. coli* expression system to obtain an efficient expression of human aromatase and raised antibodies against aromatase in rabbits. Utilizing immunohistochemistry with the resulting antibodies, we investigated the expression of CYP19 in 78 NSCLC and its association with our previous results from the investigation of other CYPs (CYP1A1, CYP2A6, CYP2E1 and CYP3A) in the same specimens (6, 14).

3. MATERIALS AND METHODS

3.1. Preparation of the human aromatase and anti-bodies

The human aromatase was previously expressed in *E. coli* DH5α using pCWori$^+$ expression vector (12, 15). We further developed the method by using pET17/BL21 (DE3) system. The cDNA insert encoding the N-terminal modified human aromatase was isolated from the plasmid NmA264RpCW (12) by the digestion with *NdeI/HindIII* and subcloned into pET17b, resulting in an aromatase expression plasmid 2cArpET. The *E. coli* BL21 (DE3) cells were cotransformed with 2cArpET and pGro12, a molecular chaperone GroEL/GroES expression plasmid (16). Four single colonies isolated from LB plates with 100 μg/ml ampicillin and 50 μg/ml kanamycin were subjected to the small-scale (25 ml) expression of aromatase. A colony best expressing aromatase was selected and stored as a glycerol stock at -80 ^{O}C for future use.

The transformed *E. coli* from glycerol stock was inoculated in 5 ml TB medium supplemented with 100 μg/ml ampicillin and 50 μg/ml kanamycin and incubated overnight at 37 ^{O}C. The overnight culture (2.5 ml) was diluted with 250 ml TB medium in a 2.8 L Fernbach flask and incubated at 37 ^{O}C for 3 hr. After the addition of 0.5 mM IPTG for the induction of T7 RNA polymerase, 1 mM δ-aminolevulinic acid (a heme precursor), 4 mg/ml L (+)-arabinose for induction of GroES/GroEL, and an additional 50 μg/ml ampicillin, the cultures were further incubated at 28 ^{O}C for approximately 20 hr until pH of the culture media reached to 7.0. Cells were harvested by centrifugation, treated with 0.5 mg/ml lysozyme in a buffer containing 50mM Tris–HCl (pH 7.2), 250mM sucrose, and 0.5mM EDTA, and proteins were extracted by sonication in buffer A (100mM potassium phosphate (pH 7.4), 500mM sodium acetate, 0.1mM EDTA, 0.1mM DTT, 20% glycerol, 1.5% sodium cholate, 1.5% Tween 20, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF)) (17). The cell lysates were ultracentrifuged, and supernatants were pooled for the purification after determination of total proteins using the BCA protein assay kit (Pierce) and P450 content by the reduced CO-difference spectrum. This new expression system of aromatase reproducibly produces more than 1000 nmol aromatase/L culture (over 50 mg/L).

The human aromatase expressed in *E. coli* was purified using Ni-NTA agarose, DEAE-Sepharose Fast Flow, and hydroxyapatite columns as described (12). The reduced CO-difference spectrum of aromatase was determined as described (18). The antibodies were raised in two rabbits using the purified aromatase from *E. coli*. The purified aromatase (0.5 mg) was emulsified with the complete Freund's adjuvant and subcutaneously injected at several sites on back of each rabbit. Two weeks later, another 0.5 mg aromatase with the incomplete Freund's adjuvant was injected for boost. Two weeks after the first and second injections, the production of antibody was determined by the standard ELISA assay using 96 well titer plates (Immuno 96 MicroWell plates, Nunc) that were coated with the purified aromatase (5 μg/ml x 100 μl/well). The diluted serum (100 μl/well) was placed in a well and incubated at 37 ^{O}C for 30 min. The HRP-conjugated goat anti-rabbit IgG (100 μl of diluted (x 5000) IgG purchased from Cappel) and o-phenylenediamine (Sigma) were used for detection. All procedures of the rabbit use were approved by Nara Medical University Animal Care and Use Committee and carried out in the university. The IgG fraction was purified from the serum using a protein A-Sepharose column, lyophilized, and stored -20 ^{O}C (19).

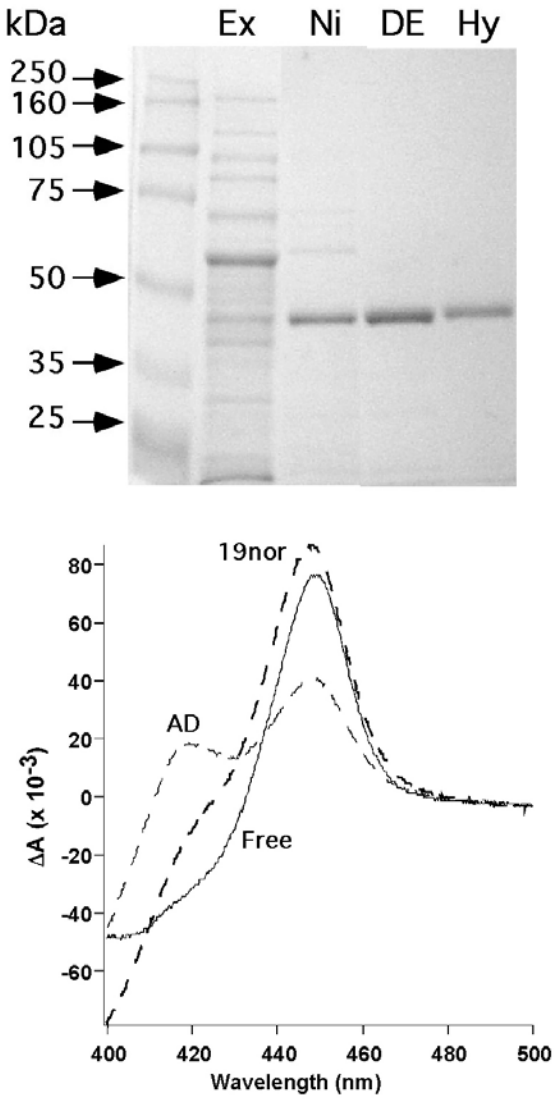

Figure 1. SDS-PAGE and reduced CO-spectra of purified CYP19 expressed in *E. coli*. Upper: SDS-PAGE was visualized with Coomassie Blue staining. Lane Ex, Extract from E. coli expressing CYP19; lane Ni, sample eluted from Ni-NTA agarose; lane DE, eluate from DEAE-Sepharose; lane Hy, purified CYP19 from hydroxyapatite. Lower: Representative reduced CO-difference spectra of the purified CYP19. Free, substrate-free form; AD, in the presence of androstenedione (0.25 mM); 19-nor, in the prsence of 19-norandrostenedione (0.25 mM).

3.2. NSCLC specimens

We examined 78 of 98 (79.6%) consecutive Japanese patients with NSCLC who underwent surgical resection at the Second Department of Surgery, School of Medicine, University of Occupational and Environmental Health, in Kitakyushu, Japan, between 1992 and 1993. The criterion for inclusion in the study was the availability of specimens and follow-up data. The median survival time (MST) of this group was 802 days. There were 56 men and 22 women ranging in age from 38 to 80 years (mean: 65.7 years). None of these patients received chemotherapy or radiotherapy prior to the operation. Twenty-six patients had stage I disease, 10 stage II, 39 stage III, and 3 stage IV, according to TNM staging (20). Histological typing of the tumors was performed according to the WHO classification (21). Resected specimens were fixed in 10% formalin for three days and were embedded in paraffin. For histological study, sections were stained with hematoxylin and eosin.

3.3. Immunohistochemical staining

Immunohistochemical staining for CYP19 was performed using the 78 NSCLC sections. The results of CYP1A1, CYP2A6, CYP2E1, and CYP3A staining from the previous publication (22) were compared with the results from the present study. CYP19 staining was performed using a standard immunoperoxidase technique. The 3 μm sections of each tumor were deparaffinized through a series of xylene and gradient ethanols to water. Slides were placed in 0.1 mol/L citrate buffer (pH 6.0), autoclaved for 10 minutes, and treated with 3% H_2O_2 for 5 minutes to reduce endogenous peroxidase activity. After the incubation with normal goat serum for 15 minutes to reduce nonspecific antibody binding, they were incubated with a 1000 dilution of primary CYP19 antibody for 40 minutes at room temperature. Histofine SAB-PO Kit (Nichirei Co., Tokyo, Japan) was used for immunoperoxidase staining according to the manufacturer's instructions. Staining was completed after 15 minutes incubation with a freshly prepared substrate-chromogen solution. We defined as CYP positive cases when more than 10% of tumor was stained (negative cases; < 10% positivity, positive cases; > 10% positivity). Statistical differences were evaluated by analysis of variance (ANOVA).

4. RESULTS

4.1. Expression, purification of human aromatase, and preparation of polyclonal antibodies

In the previous study, aromatase was expressed in *E. coli* DH5α cells using pCWori+ having the lac promoter for the induction of foreign proteins as an expression vector (12, 15). Since T7 RNA polymerase-dependent pET17b/BL21 (DE3) system has proved efficacious for the expression of foreign proteins in *E. coli*, we examined the capability of this system for the expression of human aromatase with the coexpression of molecular chaperones GroES/EL. With this expression system, human aromatase was efficiently expressed at approximately 1000-1200 nmol/L culture in 20 hr expression culture compared with 400 nmol/L culture in 40 hr expression culture from the previous report (12). The protein was purified to apparent homogeneity on SDS-PAGE (Figure 1). The purified human aromatase showed a typical reduced CO-difference spectrum with a peak at 450 nm in the absence of substrates (Figure 1). In the presence of a natural substrate androstenedione, it showed 30-40% height of the 450 nm peak with a similar height of a 420 nm-component that could be the reduced form of aromatase. Intriguingly, it showed a slightly higher peak shifted to 448 nm in the presence of an unnatural substrate 19-norandrostenedione. It was also noted that the reduced CO-complex of human aromatase was quickly (in 1-2 min) formed in the absence of substrates but very slowly (in 6-8 min) in the presence of 19-norandrostenedione.

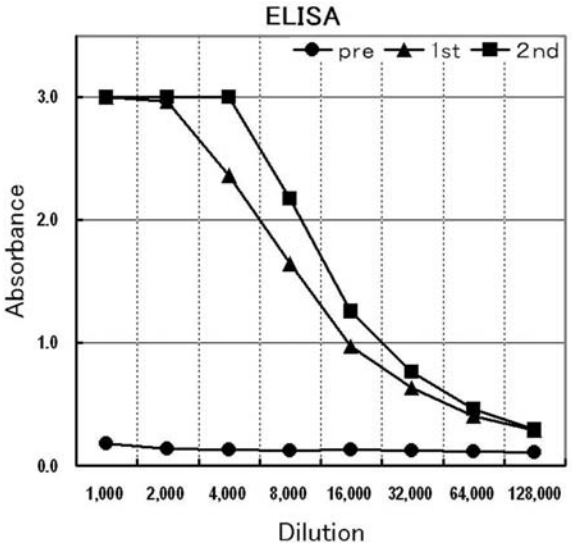

Figure 2. ELISA of anti-human aromatase sera. The production of anti-human aromatase antibody in rabbits was monitored by Enzyme-Linked Immuno-Sorbent Assay (ELISA). Blood was collected before immunization (pre), 2 weeks after immunization (1st), and 2weeks after the injection of booster (2nd). The dye formation from o-phenylenediamine by horseradish peroxidase was measured by a titer-plate reader (490 nm).

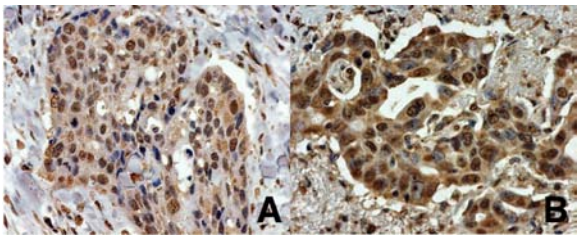

Figure 3. Immunohistochemical staining of CYP19 in NSCLC. Representative images of positive CYP19 staining (> 10% of tumor cells with specific staining) in squamous cell carcinoma (A) and adenocarcinoma (B).

Using the purified human aromatase, antibodies against human aromatase were raised in rabbits. Upon ELISA analysis, serum from a rabbit showed a high titer (Figure 2). This serum was purified on a protein A Sepharose column and the anti-aromatase IgG fraction was lyophilized and stored for a long-term storage. The purified antibody produced clear immunochistochemical images with a high signal/background ratio when they were used upon NSCLC samples (Figure 3), indicating the usefulness of the anti-human aromatase antibody produced in this study. Therefore, we used this antibody to investigate the expression of aromatase in NSCLC.

4.2. Expression of aromatase (CYP19) in NSCLC

The expression of CYP19 in non-small cell lung cancer (NSCLC) was determined by immunohistochemistry and analyzed its relationship with various clinical and pathological parameters. The relationship of CYP19 expression were also analyzed with our previous data (22) for CYP1A1, CYP2A6, CYP2E1, and CYP3A4 obtained from the same series of 78 NSCLC specimens (Table 1). Fifty (64.1%) of 78 NSCLC were stained positive for the expression of CYP19. CYP19 was abundantly and frequently expressed in both squamous cell carcinoma and adenocarcinoma (Figure 3 A and B) in contrast to the predominant expression of other CYPs in adenocarcinoma (Table 1). Although CYP1A1, 2E1, and 3A4 were more frequently expressed in female NSCLC than in male NSCLC, no significant difference in the expression of CYP19 was found between females and males. The expression of CYP19 in lung cancer was found to be unrelated also with age, smoking, cell type, N factor, stage and differentiation. However, CYP19 positive rate of T1 and T2 disease (74.5%) was significantly higher than that of T3 and T4 disease (48.3%) ($p < 0.05$). This observation could be related to that of the expression rates of CYP1A1, CYP2A6, CYP2E1, and CYP3A4 in T1 disease that were higher compared with in T2-T4 disease, although the expression of these CYPs were only in adenocarcinoma. In addition, there was no trend indicating that the CYP19 expression in NSCLC is associated with poor survival (data not shown).

4.3. Correlation between CYP19 and other CYPs for the expression in NSCLC

Since CYP3A family members and CYP1A1 are involved in the metabolism of estrone and estradiol (23), the expression of these CYPs might be correlated with the expression of CYP19 in NSCLC. Therefore, the relationship of CYP19 expression with other CYPs was analyzed (Table 2). However, the expression of CYP19 was independent of the expression of other CYPs.

4.4. Mutations in p53 and the frequency of CYP19 expression

Since aberrant expression and polymorphisms of enzymes involved in drug metabolism may be associated with p53 mutations and involved in the poor prognosis (24), we previously investigated the same specimens and determined that forty-one of 78 NSCLC (52.6%) expressed detectable amounts of p53 protein, and that 29 of 78 (37.2%) expressed mutant *p53* by PCR-SSCP method (22). Therefore, we analyzed the association of aromatase expression and p53 mutations in NSCLC using the previous data and the data from this study. As seen in Table 3, 26 specimens (52%) among 50 aromatase positive specimens expressed detectable amounts of p53. Similarly, 15 (53.6%) among 28 aromatase negative specimens expressed the p53 protein. We found no relationships between p53 mutations and the frequency of CYP19 (Table 3) as no relationship was found between mutations of p53 and the other CYP expression. Therefore, we concluded that the expression of CYP19 is not correlated with mutations of p53.

5. DISCUSSION

In this study, we developed the expression method of human aromatase (CYP19) in *E. coli* using pET17/BL21 (DE3) system with the coexpression

Table 1. Relationships of the immunopositivity of CYP19 with other P450s (CYP1A1, CYP2A6, CYP2E1, and CYP3A4) and various clinical or pathological parameters in NSCLC patients

Parameter		CYP1A1[6]			CYP2A6[6]			CYP2E1[6]			CYP3A[6]			CYP19		
	n	+	-	p	+	-	p	+	-	p	+	-	p	+	-	p
Sex																
Female	22	13 (59.1)	9		9 (40.9)	13		12 (54.5)	10		11 (50.0)	11		14 (63.6)	8	
Male	56	8 (14.3)	48	**<0.01**	13 (23.2)	43	0.199	7 (12.5)	49	**<0.01**	8 (14.3)	48	**<0.01**	36 (64.3)	20	**1.0**
Age																
≤ 65	37	8 (21.6)	29		8 (21.6)	29		8 (21.6)	29		6 (16.2)	31		25 (67.6)	12	
> 65	41	13 (31.7)	28	0.46	14 (34.1)	27	0.33	11 (26.8)	30	0.79	13 (31.7)	28	0.18	25 (61.0)	16	0.71
Smoking [1]																
< 40	42	16 (38.1)	26		15 (35.7)	27		14 (33.3)	28		14 (33.3)	28		25 (59.5)	17	
≥ 40	36	5 (13.9)	31	**<0.05**	7 (19.4)	29	0.18	5 (13.9)	31	0.08	5 (13.9)	31	0.08	25 (69.4)	11	0.50
Cell type																
Ad [2]	48	21 (43.8)	27		22 (45.8)	26		19 (39.6)	29		19 (39.6)	29		29 (60.4)	19	
Sq [3]	30	0 (0.0)	30	**<0.01**	0 (0.0)	30	**<0.01**	0 (0.0)	30	**<0.01**	0 (0.0)	30	**<0.01**	21 (70.0)	9	0.54
T [4]																
1	21	12 (57.1)	9		10 (47.6)	11		11 (52.4)	10		11 (52.4)	10		13 (61.9)	8	
2	26	4 (15.4)	22		4 (15.4)	22		3 (11.5)	23		4 (15.4)	22		22 (84.6)	4	
3, 4	31	5 (16.1)	26	**<0.01**	8 (25.8)	23	**<0.05**	5 (16.1)	26	**<0.01**	4 (12.9)	27	**<0.01**	15 (48.3)	16	**<0.05**
N [5]																
0	36	17 (47.2)	19		13 (36.1)	23		13 (36.1)	23		15 (41.7)	21		22 (61.1)	14	
1, 2, 3	42	4 (9.5)	38	**<0.01**	9 (21.4)	33	0.24	6 (14.3)	36	**<0.05**	4 (9.5)	38	**<0.01**	28 (66.7)	14	0.78
Stage																
I	26	15 (57.7)	11		12 (46.2)	14		12 (46.2)	14		14 (53.8)	12		18 (69.2)	8	
II - IV	52	6 (11.5)	46	**<0.01**	10 (19.2)	42	**<0.05**	7 (13.5)	45	**<0.01**	5 (9.6)	47	**<0.01**	32 (61.5)	20	0.68
Differentiation																
Well	4	3 (75.0)	1		2 (50.0)	2		3 (75.0)	1		3 (75.0)	1		2 (50.0)	2	
Moderately	52	16 (30.8)	36		16 (30.8)	36		14 (26.9)	38		15 (28.8)	37		34 (65.4)	18	
Poorly	22	2 (9.1)	20	**<0.05**	4 (18.2)	18	0.33	2 (9.1)	20	**<0.05**	1 (4.5)	21	**<0.01**	14 (63.6)	8	0.83
Total	78	21 (26.9)	57		22 (28.2)	56		19 (24.4)	59		19 (24.4)	59		50 (64.1)	28	

Smoking [1]; the amount of lifetime cigarette consumption (pack-year) <40; low level smoker, >40; high level smoker, Ad [2]; Adenocarcinoma, Sq [3];Squamous cell carcinoma, T [4]; Tumor stage, N [5]; Nodal stage, [6]Data from the previous report (22).

Table 2. Relationships of CYP19 expression with the expression of CYP1A1, CYP2A6, CYP2E1, and CYP3A in NSCLC patients

	Number of positive cases	**CYP1A1[1]**	**CYP2A6[1]**	**CYP2E1[1]**	**CYP3A[1]**	**CYP19**
CYP1A1	21		<0.05	<0.01	<0.01	0.61
CYP2A6	22			0.17	0.17	0.75
CYP2E1	19				<0.01	1.0
CYP3A	19					1.0
CYP19	50					

Upper right; p-values for co-expression of CYP1A1, CYP2A6, CYP2E1, Cyp3A and CYP19 expression.
[1]data from Oyama *et al.* (22).

Table 3. Relationships between p53 alterations and CYP19 expression

		p53 expression[1] (%)	**p53 mutation[1] (%)**				
factor	n	+	-	p	+	-	p
CYP19							
positive	50	26 (52.0)	24		22 (44.0)	28	
negative	28	15 (53.6)	13	1.0	7 (25.0)	21	0.16
Total	78	41 (52.6)	37		29 (37.2)	49	

[1]The data of expression and mutation for p53 were from the previous publication (6).

of molecular chaperones GroES/GroEL. The newly developed expression method was very efficient (>1000 nmol/l culture) compared with the previous method (12), which allowed us to obtain quantities of purified CYP19. The purified CYP19 showed a typical reduced CO-difference spectrum with a peak at 450 nm (Figure 1). Intriguingly, this enzyme showed a lower 450 nm peak with a large peak at 420 nm in the presence of androstenedone. Since this 420 nm peak quickly appears and decreases along with the increase of the 450 nm peak, the 420 nm peak may

be produced from the reduced form of CYP19 but not the spectrum from the inactive P420 form. In contrast, 19-norandrostenedione-bound form showed a stable, reduced CO-difference spectrum with a peak shifted to 448 nm. The results suggest that the steric hindrance caused by 19-methyl group of androstenedione inhibits the formation of CO-complex of CYP19. The reactions mediated by atypical P450s, allene oxide synthase (25) and thromboxane synthase (26) that do not require molecular oxygen (O_2) nor electrons from reductase, are not inhibited by carbon monoxide (CO). However, mono-oxygenation reactions of typical P450s requiring O_2 and the electron donor are inhibited by CO, with the exception of the aromatization reaction. Using placental microsomes, Meigs and Ryan (27) reported that aromatase activities were not inhibited by carbon monoxide when androstenedione was used as a substrate and that the aromatization of 19-norandrostenedione was inhibited by carbon monoxide. Tosha *et al.* (28) also reported that the 450 nm peak of aromatase was decreased in the presence of androstenedione and testosterone but not in the presence of 19-norandrostenedione. Our results support the previous reports and provide the first example of the reduced CO-difference spectra of human aromatase showing the clear inhibition of CO-binding by androstenedione.

Utilizing anti-aromatase antibody produced in this study, we determined the expression of CYP19 in NSCLC specimens from Japanese patients. CYP19 was expressed at a high frequency (64%) in NSCLC from Japanese patients (Table 1), although the frequency was lower than 86% in the NSCLC from Caucasian patients (13). We did not found the gender dependence in frequency of CYP19 expression, which is also consistent with the results by Weinberg *et al.* (13). Although the relationships of the expression of CYP19 with clinical and pathological parameters were analyzed, the expression of CYP19 in NSCLC was independent of other factors except with the tumor stage (Table 1). CYP1B1 as well as CYP1A1 and CYP3A5 convert 17β-estradiol to 4-hydroxyestradiol, a carcinogenic derivative in animal models (29-31). CYP1A1 and CYP3A4 catalyze the conversion of 17-estradiol to 2-hydroxyestradiol, an inactive estrogen derivative (32). Therefore, the expression of these CYP enzymes in NSCLC might be correlated with the expression of CYP19. Although we expected a correlation of CYP1A1 and CYP3A with CYP19 expression, we did not find any correlation of CYP19 expression with other CYPs in NSCLC.

Utilizing a xenograph model system, Mah *et al.* reported the stimulatory effect of aromatase and estrogens on tumor growth (33). Therefore, therapeutic targeting to block estrogen signaling pathway may provide new options for the treatment of NSCLC patients (10, 13). Hormonal treatment, such as tamoxifen, could be useful for NSCLC patients. Tamoxifen binds to the estrogen receptor and blocks the estrogen function. Therefore, tamoxifen is used for the treatment of estrogen receptor-positive breast cancer (34) and also used as a preventative agent in women who are at an increased risk of developing breast cancer (35). Several CYP enzymes are expressed in NSCLC and involved in activation and/or inactivation of drugs used for the management of the cancer (10, 23). Of the CYP enzymes, CYP3A4 is capable of catalyzing the 4-hydroxylation of tamoxifen, yielding 4-hydroxytamoxifen that is a potent anti-estrogen with high affinity for the estrogen receptor. Therefore, tamoxifen could be more effective for the treatment of CYP19-positive NSCLC when it expresses CYP3A4. Alternatively, third generation of aromatase inhibitors, anastrozole, letrozole, and exemestane, have proved very efficacious in management of hormone-dependent breast cancer in post-menopausal women and also in prevention of recurrence. The aromatase inhibitors have also proved superior to tamoxifen for the treatment of breast cancer. Therefore, post-menopausal women with aromatase-positive NSCLC tumor might be adequate candidates for the targeted treatment with aromatase inhibitors. Since aromatase (CYP19) is expressed in various tissues and organs and estrogens stimulate growth of many cell types, some other cancers could be also estrogen-dependent with the CYP19 expression. If so, more cancers might be managed by aromatase inhibitors.

6. ACKNOWLEDGEMENT

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Abbreviations: CYP: cytochrome P450, NSCLC: non-small cell lung cancer, ER: estrogen receptor, ERT: estrogen replacement therapy, PgR: progesterone receptor

Key Words: Aromatase, CYP19, Cytochrome P450, CYP, Non-Small Cell Lung Cancer, NSCLC, Estrogen Replacement Therapy, ERT, E. coli, Aromatase Inhibitors, Estrogen Receptor, ER, Progesterone Receptor, PgR, Survival

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