Molecular and Cellular Endocrinology 392 (2014) 136–143

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Tissue concentrations of estrogens and aromatase immunolocalization in interstitial pneumonia of human lung

Shinji Taniuchi [a], Fumiyoshi Fujishima [a], Yasuhiro Miki [b,c], Keiko Abe [d], Yasuhiro Nakamura [b], Satoko Sato [a], Atsuko Kasajima [a], Misaki Fue [c], Kazuyuki Ishida [e], Mika Watanabe [a], Tomohiro Sakakibara [f], Sumiko Maeda [g], Takashi Suzuki [h,*], Hironobu Sasano [a,b]

[a] Departments of Pathology, Tohoku University, Graduate School of Medicine, Sendai, Japan
[b] Departments of Anatomic Pathology, Tohoku University, Graduate School of Medicine, Sendai, Japan
[c] Department of Disaster Obstetrics and Gynecology, International Research Institute of Disaster Science, Tohoku University, Sendai, Japan
[d] Department of Pathology, Saitama Medical Center, Saitama Medical University, Saitama, Japan
[e] Division of Diagnostic Molecular Pathology, Department of Pathology, School of Medicine, Iwate Medical University, Iwate, Japan
[f] Departments of Respiratory Medicine, Tohoku University, Graduate School of Medicine, Sendai, Japan
[g] Departments of Thoracic Surgery, Tohoku University Hospital, Sendai, Japan
[h] Departments of Pathology and Histotechnology, Tohoku University, Graduate School of Medicine, Sendai, Japan

ARTICLE INFO

Article history:
Received 9 December 2013
Received in revised form 18 April 2014
Accepted 16 May 2014
Available online 23 May 2014

Keywords:
Aromatase
Estrogen
Interstitial pneumonia
Lung

ABSTRACT

Interstitial pneumonia (IP) is characterized by various degrees of pulmonary fibrosis and inflammation. Estrogens have been demonstrated to play important roles in physiological and pathological conditions of human lung, but significance of estrogens has remained unknown in human IP. Therefore, we measured estrogen concentrations and immunolocalized aromatase and estrogen receptor β (ERβ) in IP tissues. Estradiol concentration was significantly (2.8-fold) higher in IP than normal lung tissues, and aromatase activity evaluated by estradiol/testosterone ratio was also significantly (7.2-fold) elevated in IP tissues. Aromatase immunoreactivity in alveolar epithelial cells was significantly frequent in IP than normal lung or inflammatory lung disease other than IP, and it was positively associated with ERβ immunoreactivity in these cells of IP. These results suggest that estradiol concentration is locally increased in human IP tissue by aromatase, and increased estrogens may play an important role in the development of IP through ERβ in the alveolar epithelial cells.

1. Introduction

Interstitial lung disease, or diffuse parenchymal lung disease, is a heterogeneous group of a pulmonary disorder which shares similar clinical, radiological or pathological features with miscellaneous etiologies, such as drug toxicity, collagen vascular diseases, occupational/environmental exposures and other unknown causes (American Thoracic Society and European Respiratory Society, 2001; King, 2005; Ryu et al., 2007). A great majority of interstitial lung disease is associated with some features of interstitial pneumonia (IP), which is characterized by various degrees of pulmonary fibrosis and inflammation (American Thoracic Society and European Respiratory Society, 2001; King, 2005; Ryu et al., 2007). The mechanisms of pulmonary fibrosis have been gradually clarified in recent years (Selman and Pardo, 2006; Willis et al., 2006; Strieter and Mehrad, 2009), but several subtypes of IP, including the idiopathic pulmonary fibrosis, are still associated with poor clinical outcome and high mortality rate despite the advent of therapy (Raghu et al., 2011). Therefore, it has become very important to examine the details of biological features of IP and to develop the targeted therapies aimed at the specific factors involved in its biological behaviors.

Estrogens have been recently demonstrated to be involved in various physiological and/or pathological functions in many human organs other than their classical target tissues, through binding to estrogen receptor (ER) (Gustafsson, 1999; Morani et al., 2008; Carey et al., 2007). ER consists of ERα and ERβ in human. Human lung tissue expresses both ERα and ERβ and show biological responses to estrogen (Stabile et al., 2002), and its predominant subtype is considered ERβ (Couse et al., 1997; Taylor and Al-Azzawi, 2000; Omoto et al., 2001). Estrogens have been also

* Corresponding author. Address: Department of Pathology and Histotechnology, Tohoku University, Graduate School Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan. Tel./fax: +81 22 717 8677.
E-mail address: t-suzuki@patholo2.med.tohoku.ac.jp (T. Suzuki).

http://dx.doi.org/10.1016/j.mce.2014.05.016
0303-7207/

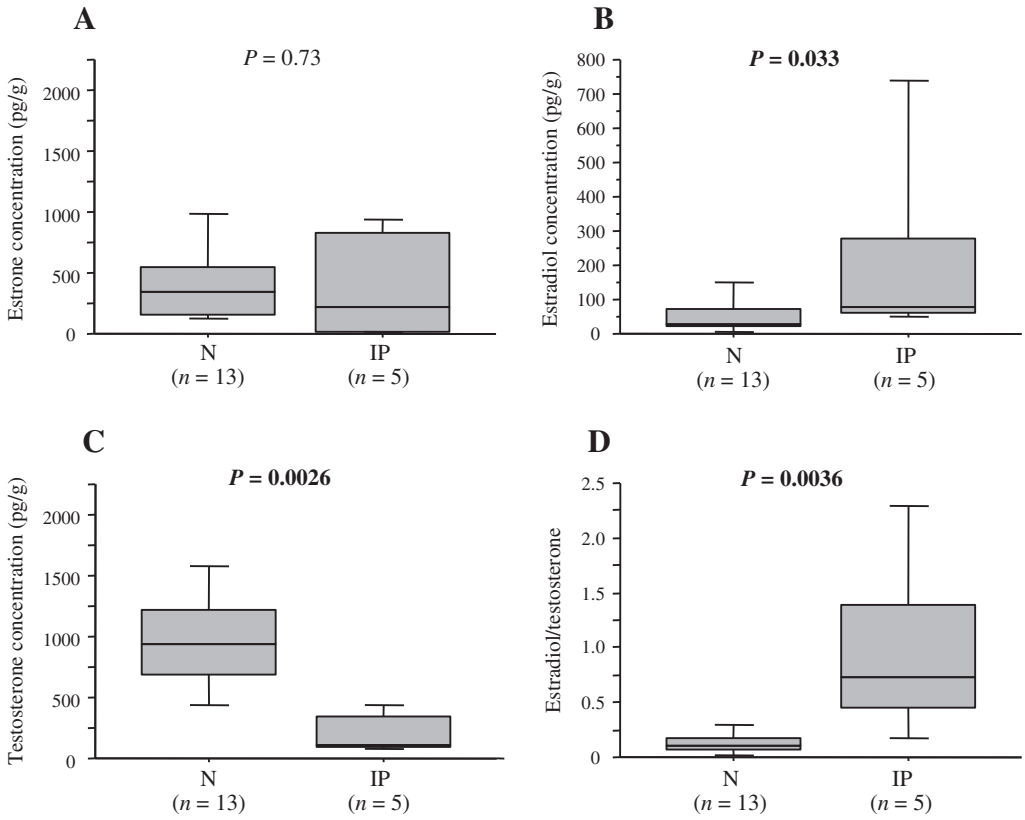

Fig. 1. Tissue concentrations of estrone (A), estradiol (B), testosterone (C) and estradiol/testosterone ratio (D) in each specimens of IP using LC–MS/MS analysis. Data are represented as box and whisker plots. The median value was represented by a horizontal line in the box pot, and gray box denoted the 75th (upper margin) and 25th percentiles of the values (lower margin), respectively. The upper and lower bars indicated the 90th and 10th percentiles, respectively. The statistical analysis was performed using a Wilcoxon test. *P*-value less than 0.05 was considered significant, and described as boldface. N: normal lung tissue with no significant pathological abnormalities, and IP: interstitial pneumonia.

well demonstrated to be locally produced from circulating inactive steroids by aromatase, a rate limiting key enzyme in estrogen biosynthesis converting androgens to estrogens. Increased local estrogen actions through aromatase and ER are closely associated with a variety of pathological conditions, such as breast carcinoma (Miki et al., 2007), hepatocellular carcinoma (Castagnetta et al., 2003; Vizoso et al., 2007), atherosclerosis (Murakami et al., 2001; Nakamura et al., 2003), diabetic nephropathy (Prabhu et al., 2010), Alzheimer disease (Ishunina et al., 2007), rheumatoid arthritis (Ishizuka et al., 2004; Schmidt et al., 2005), and skin wound healing (Ashcroft et al., 1999; Mills et al., 2005; Gilliver et al., 2007; Merlo et al., 2008).

Estrogens have been demonstrated to influence pulmonary development and physiology (Carey et al., 2007), and ERβ contributes to the maintenance of normal lung tissue functions (Patrone et al., 2003). In addition, biologically active estrogen, estradiol, significantly increased cell proliferation of ERβ-positive lung carcinoma cells, and tissue concentrations of estradiol were also reported to be elevated in lung carcinoma tissues (Niikawa et al., 2008). The intratumoral estradiol concentration of lung carcinoma was reported to be positively associated with intratumoral aromatase expression (Niikawa et al., 2008), and aromatase immunoreactivity was associated with worse prognosis in women with lung carcinoma (Mah et al., 2007). Therefore, estrogens and/or aromatase become possible new therapeutic targets of human lung cancer patients. Also, expression of other sex hormone progesterone receptor (PR) has been reported in lung carcinoma (Ishibashi et al., 2005).

Effects of sex steroids upon IP have been examined mostly in rodent models of the disease (Gharaee-Kermani et al., 2005; Voltz et al., 2008; Brass et al., 2010), and profibrotic effects of estrogens were clearly demonstrated in these models (Gharaee-Kermani et al., 2005). However, to the best of our knowledge, estrogen concentration and aromatase expression have not been previously reported in actual human IP tissues, and therefore significance of estrogens remains largely unknown in human IP. Therefore, in this study, we examined the concentration of sex steroids and immunoreactivity of aromatase and ERβ in human IP tissues, and compared the results with those in non-pathological lung tissue in order to evaluate a possible involvement of estrogenic actions and *in situ* production of estrogens in human IP.

2. Materials and methods

2.1. Patients and tissues

Snap-frozen specimens of 5 IP lesions of the human lung were retrieved from autopsy files at Tohoku University Hospital, Sendai, Japan, to measure tissue concentrations of sex hormones. These samples were obtained from five men who died of IP [median age 76 (range 61–80) yrs]. As for the controls, we also used 13 snap-frozen specimens of non-pathologic male lung tissue which were obtained from 10 patients [median age 67 (range 45–82) yrs] who underwent lung lobectomy due to primary lung carcinoma in the Department of Thoracic Surgery, Tohoku University Hospital and 3 autopsy cases at Tohoku University Hospital, Sendai, Japan. Their age and cause of death were as follows: 65 yrs (pancreatic cancer), 67 yrs (cardiac failure) and 87 yrs (prostatic cancer).

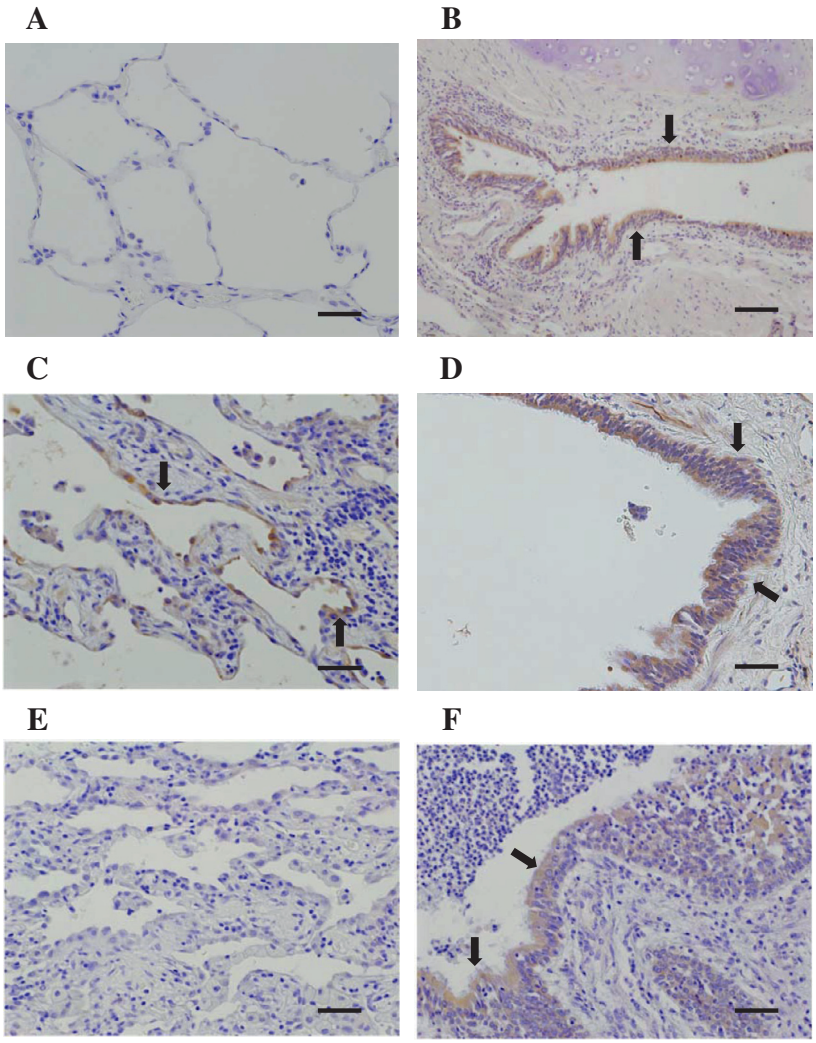

Fig. 2. Aromatase immunolocalization in IP cases. Aromatase immunoreactivity was negative in AEC (A) and positive in BEC (B) in normal human lung. Aromatase immunoreactivity was detected both in AEC (C) and BEC (D) of IP tissue. Aromatase immunoreactivity was negative in AEC (E) and positive in BEC (F) in other inflammatory lung diseases. Arrows showed aromatase-positive epithelium. Bar = 50 μm, respectively.

Table 1
Immunoreactivity for aromatase in lung epithelial cells.

Cell types	Immunoreactivity	*N*	IP	ID	*P*-value	
		(*n* = 21)	(*n* = 33)	(*n* = 23)	*N vs* IP	IP *vs* ID
AEC	+	0 (0%)	22 (67%)	1 (4%)		
	–	21 (100%)	11 (33%)	22 (96%)	**<0.0001**	**<0.0001**
BEC	+	19 (90%)	27 (82%)	21 (92%)		
	–	2 (10%)	6 (18%)	2 (8%)	0.38	0.29

Data are presented as the number of cases and percentage in each group. *P*-values were evaluated by a cross-table using the chi-square test between *N* and IP and between IP and ID. *P*-values < 0.05 were considered significant, and are shown in bold. *N*, normal lung; IP, interstitial pneumonia; ID, other inflammatory diseases; AEC, alveolar epithelial cell; BEC, bronchiolar epithelial cell.

Another set of lung tissue specimens was obtained by partial lung resection (*n* = 77) at Tohoku University Hospital, Sendai, Japan, from 2003 to 2013, to perform immunohistochemical evaluation. Among these cases, 33 cases were derived from the patients with IP [22 men and 11 women, median age 63 (range 24–81) yrs]. They were histopathlogically classified as follows: usual interstitial pneumonia (UIP) pattern (*n* = 21), nonspecific interstitial pneumonia (NSIP) pattern (*n* = 5) and organizing pneumonia (OP) pattern (*n* = 7). Twenty-three cases [17 men and 6 women, median age 61 (range 19–80) yrs] were derived from inflammatory lung diseases other than IP, such as infectious diseases (*n* = 10) [*i.e.*, histologically consistent with granulomatous inflammation (*n* = 9), bronchopneumonia (*n* = 1)] and nonspecific chronic bronchiolitis (*n* = 13). Other twenty-one cases [13 men and 8 women, median age 57 (range 18–67) yrs] were pulmonary tissues associated with no significant histological abnormalities obtained from the patients undergoing partial lung resection for localized primary lung carcinoma (*n* = 4), metastatic lung tumor (*n* = 10), bulla (*n* = 6), and lung tissue adjacent to an intrapulmonary lymph node (*n* = 1). All the specimens had been fixed with 10% formalin at room temperature and embedded in paraffin wax.

In this study, the degree of fibrosis was also histologically classified into two groups based on the modified method of Ashcroft et al. (1988). Ashcroft grades 4 and 5 were categorized as mild, while the grades 6 and 7 as moderate to severe. No IP cases corresponded to Aschcroft grades 0–3 in our present study. The degree of inflammation was classified into two groups according to the report of Fulmer et al. (1979).

Research protocols for this study were approved by the Ethics Committee at Tohoku University School of Medicine (2009–204).

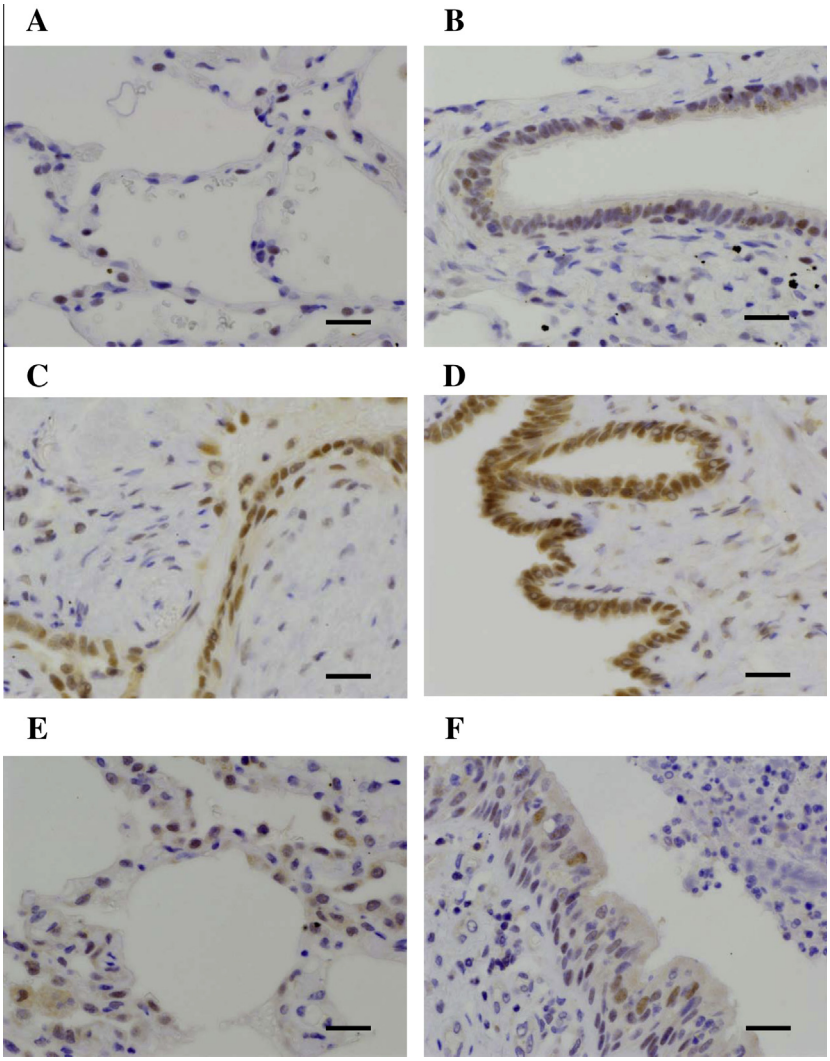

Fig. 3. ERβ immunoreactivity in IP cases. ERβ immunoreactivity was positive both in AEC (A) and BEC (B) of normal human lung, IP (C and D, respectively) and ID (E and F, respectively) tissues. Bar = 20 μm, respectively.

2.2. Liquid chromatography/electrospray tandem mass spectrometry (LC–MS/MS)

Tissue concentrations of estrone, estradiol and testosterone were measured by LC–MS/MS analysis, in ASKA Pharma Medical (Kawasaki, Japan), as described previously (Miki et al., 2007; Suzuki et al., 2007; Niikawa et al., 2008). Briefly, lung tissue specimens were homogenized in 1 ml of distilled water. After addition of 100 pg of estrone–$^{13}C_4$ and estradiol–$^{13}C_4$ (Hayashi Pure Chemical Industries, Osaka, Japan), and testosterone–$^{2}H_3$ (CDN Isotope Inc., Quebec, Canada) as internal standards, steroids were extracted with diethyl ether from the homogenate. In this study, we used liquid chromatography (Agilent 1100; Agilent Technologies, Waldbronn, Germany) coupled with an API 4000 triple-stage quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada) operated with electron spray ionization in the positive-ion mode, and the chromatographic separation was performed on Cadenza CD-C_{18} columns (Imtakt, Kyoto, Japan).

2.3. Immunohistochemistry

Immunohistochemistry was performed by labeled streptavidin biotin method using a Histofine kit (Nichirei, Tokyo, Japan). Primary antibodies used in this study were mouse monoclonal antibodies against aromatase (clone #677/H7) (Niikawa et al., 2008; Abe et al., 2010), ERα (clone 6F11; Leica Biosystems, St Louis, Mo, USA), ERβ (clone 14C8; GeneTex Inc., San Antonio, TX, USA) and PR (clone 1A6; Abcam, Cambridge, UK). Antigen retrieval was performed by autoclaving the slides in citrate buffer (0.01 mol/L) at 121 °C for 5 min. These slides were then immersed in 3,3′-diaminobenzidine solution in order to visualize antigen–antibody complexes. As for the positive controls of immunostaining, we used tissues of human placenta for aromatase and prostatic carcinoma specimens for ERβ (Abe et al., 2010). As for the negative control of immunostaining, normal mouse IgG was used instead of the primary antibodies in this study.

The immunoreactivity was independently evaluated by 3 different pathologists (S.T., K.A., and F.F.) who were blinded to the clinicopathological findings of the cases. When there was a disagreement among the observers, immunoreactivity was reassessed through multi-headed light microscopy.

2.4. Statistical analysis

Statistical analyses were performed by Wilcoxon test and Pearson chi-square test using JMP Pro version 9.02 (SAS Institute Inc., Cary, NC, USA). *P*-value less than 0.05 was considered to be significant.

Table 2
ERβ immunoreactivity in lung epithelilal cells.

Cell types	Immunoreactivity	*N*	IP	ID	*P*-value	
		(*n* = 21)	(*n* = 33)	(*n* = 23)	*N vs* IP	IP *vs* ID
AEC	+	16 (76%)	30 (91%)	15 (65%)		
	–	5 (24%)	3 (9%)	8 (35%)	0.14	**0.022**
BEC	+	20 (95%)	31 (94%)	18 (78%)		
	–	1 (5%)	2 (6%)	5 (21%)	0.84	0.093

Data are presented as the number of cases and percentage in each group. *P*-values were evaluated by a cross-table using the chi-square test between N and IP and between IP and ID. *P*-values < 0.05 were considered significant, and are shown in bold. N, normal lung; IP, interstitial pneumonia; ID, other inflammatory diseases; AEC, alveolar epithelial cell; BEC, bronchiolar epithelial cell.

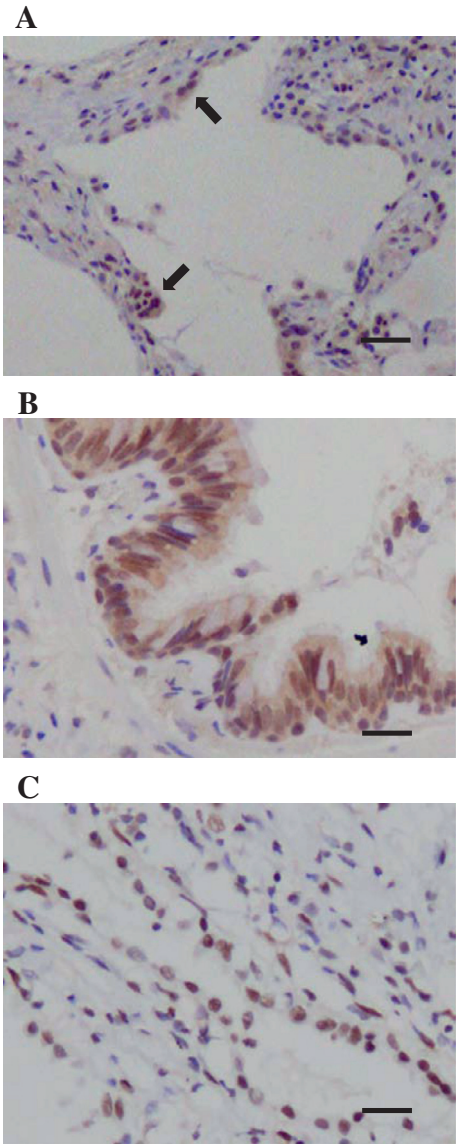

Fig. 4. ERα and PR immunolocalization in lung tissues. ERα immunoreactivity was detected in AEC (A) (arrows) and BEC (B) of IP tissue, and PR was positive in AEC (C) of ID case. Bar = 20 μm, respectively.

3. Results

3.1. Concentrations of sex steroids in IP tissues

We first examined tissue concentrations of sex steroids in IP and normal lung tissues using LC–MS/MS. The median (minimum–maximum) value of estrone was 225 (12–934) pg/g in IP, and was not significantly different ($P = 0.73$) from that in normal lung tissues examined [340 (97–1,994) pg/g] (Fig. 1A). However, the tissue estradiol concentration was significantly higher ($P = 0.033$ and 2.8-fold) in IP [79 (51–741) pg/g] than that in the normal lung [28 (4–368) pg/g] (Fig. 1B). The testosterone level in IP tissue was significantly lower ($P = 0.0026$ and 0.12-fold) in IP [115 (71–433) pg/g] than that in the normal lung [943 (246–1,974) pg/g] (Fig. 1C). The corresponding estradiol/testosterone ratio in each specimen was significantly higher ($P = 0.0036$ and 7.2-fold) in IP than normal lung tissues (Fig. 1D).

3.2. Immunolocalization of aromatase and ERβ in IP

We then immunolocalized aromatase and ERβ in normal lung, IP, and inflammatory lung disease other than IP. Aromatase immunoreactivity was negative in alveolar epithelial cells (AEC) of normal lung (Fig. 2A), while weakly positive in the bronchiolar epithelial cells (BEC) (Fig. 2B). Aromatase immunoreactivity was, however, frequently detected both in AEC (Fig. 2C) and BEC (Fig. 2D) in IP tissues. In other inflammatory lung disease, aromatase immunoreactivity was negligible in AEC (Fig. 2E), although weakly positive in BEC (Fig. 2F). An association of aromatase immunoreactivity between normal lung and IP or between IP and other inflammatory disease was summarized in Table 1. Aromatase immunoreactivity was significantly more frequent in IP than normal lung or other inflammatory disease in AEC ($P < 0.0001$, respectively), but not in BEC.

ERβ immunoreactivity was frequently detected both in AEC and BEC of the normal lung (Fig. 3A and B), IP (Fig. 3C, 3D) and other inflammatory disease (Fig. 3E and F). As summarized in Table 2, ERβ immunoreactivity in AEC of IP was significantly higher ($P = 0.022$) than that of the other inflammatory disease. ERβ immunoreactivity in BEC of IP was not significantly different from that of normal lung ($P = 0.84$) or other inflammatory disease ($P = 0.093$) in this study.

The anti-ERβ antibody used in this study (clone 14C8) recognizes N-terminus of ERβ which is conserved in ERβ isoforms 1, 2 and 5 (Skliris et al., 2008). When we tested other anti-ERβ antibody available for paraffin-embedded tissues (clone PPG5/10; AbD Serotec, Oxford, UK) which recognizes C-terminus of ERβ and detects ERβ1 isoform (Skliris et al., 2002; Skliris et al., 2008), similar immunolocalization was detected in the normal lung, IP and ID tissues (Supplementary Fig. S1A–F).

On the other hand, ERα immunoreactivity was positive only in one IP tissue (both AEC (Fig. 4A) and BEC (Fig. 4B)), and PR was immunolocalized only in one ID case (AEC (Fig. 4C)) among the 77 cases examined.

3.3. Aromatase in IP

As summarized in Table 3, aromatase immunoreactivity in AEC was significantly higher ($P = 0.021$) in UIP pattern, and positively

associated with degree of fibrosis ($P = 0.021$), ERβ immunoreactivity in AEC ($P = 0.010$) and ERβ immunoreactivity in BEC ($P = 0.039$) in IP cases. The status of aromatase immunoreactivity was not significantly associated with other clinicopathological parameters, such as patients' age, sex or degree of inflammation. Aromatase immunoreactivity in BEC was not significantly associated with any clinicopathological parameters examined.

4. Discussion

This is the first study to demonstrate tissue concentrations of sex steroids in human IP tissues, to the best of our knowledge. In this study, estradiol level was significantly higher (2.8-fold) in IP than in normal lung tissues (Fig. 1B), and estradiol/testosterone ratio in each specimen, which is considered to be correlated with aromatase activity (Cakan et al., 2009), was also significantly higher (7.2-fold) in IP than in normal lung (Fig. 1D). Significantly higher estrogen concentration in the tissues associated with increased aromatase expression has been reported in pathological conditions of some organs, such as the breast, liver, joint and skin. As for pulmonary disease, Niikawa et al. (2008) reported that intratumoral estradiol concentration was significantly higher in the lung carcinoma than in corresponding non-neoplastic lung tissues, and the estradiol concentration in the lung carcinoma was closely associated with intratumoral aromatase expression. Therefore, estradiol level is considered to be locally elevated in human IP tissues by aromatase.

In our present study, aromatase immunoreactivity in AEC was frequently ($P < 0.0001$) detected in IP, whereas negative in the normal lung tissue. Previous studies demonstrated the relatively low levels of aromatase mRNA (Demura et al., 2011) and enzymatic activity (Weinberg et al., 2005) in normal lung tissues, and aromatase was also reported to be immunolocalized in the BEC but not in AEC (Mah et al., 2007; Mah et al., 2011), which is consistent with results of our present study. In addition, aromatase immunoreactivity in AEC was negligible in inflammatory lung disease other than IP as shown in Table 1. Therefore, markedly increased aromatase in AEC of IP could be considered to result in increased local estradiol concentration of the IP lung tissues.

ERβ has been reported as the predominant subtype of ER in human lung, and to be detected in both AEC and BEC (Couse et al., 1997; Taylor and Al-Azzawi, 2000; Omoto et al., 2001). Results of our present study demonstrated that ERβ immunoreactivity was detected both in AEC and BEC of the normal lung, IP and other inflammatory disease. In addition, ERβ immunoreactivity was more frequently detected in IP than in normal lung or other inflammatory disease (Table 2), and both ERβ and aromatase immunoreactivity was significantly associated in AEC of IP tissues (Table 3). Highly concordant co-expression of aromatase and ERβ was also reported in human lung carcinoma (Abe et al., 2010). These results suggest that estrogen action is mainly mediated in AEC through aromatase and ERβ in human IP tissues. However, number of IP cases negative for ERβ immunoreactivity in AEC was very limited ($n = 3$) in this study, further examination with a larger sample set is needed to confirm the co-expression of aromatase and ERβ in AEC of IP tissues.

Clinical and biological significance of estrogen actions has largely remained unclear in human IP, but profibrotic effects of estrogens have been reported in rodent models of some pulmonary diseases. Gharaee-Kermani et al. (2005) demonstrated that degrees of fibrosis were higher in female rats than in male rats in the analysis of the bleomycin (BLM)-induced pulmonary fibrosis models, and that both lung hydroxyproline contents and procollagen mRNA were significantly decreased in the BLM treated ovariectomized rats with subsequent recovery by exogenous estradiol supplementation. Results of tissue remodeling in bronchial asthma models also demonstrated that female mice were significantly associated with more abundant collagen deposition in airways and alveolar

Table 3
Aromatase immunoreactivity in interstitial pneumonia ($n = 33$).

Parameters	AEC			BEC		
	+ ($n = 22$)	– ($n = 11$)	*P*-value	+ ($n = 27$)	– ($n = 6$)	*P*-value
Age						
>60	13 (39%)	6 (18%)		17 (52%)	2 (6%)	
≦60	9 (27%)	5 (15%)	0.80	10 (30%)	4 (12%)	0.18
Sex						
Male	13 (39%)	9 (27%)		17 (52%)	5 (15%)	
Female	9 (27%)	2 (6%)	0.19	10 (30%)	1 (3%)	0.34
Histological feature						
UIP pattern	17 (52%)	4 (12%)		19 (56%)	2 (6%)	
Others	5 (15%)	7 (21%)	**0.021**	8 (24%)	4 (12%)	0.088
Degree of fibrosis[*]						
Mild	5 (15%)	7 (21%)		7 (23%)	4 (12%)	
Moderate to severe	17 (52%)	4 (12%)	**0.021**	18 (58%)	2 (6%)	0.19
Degree of inflammation[**]						
Minimal to mild	3 (9%)	4 (12%)		8 (24%)	4 (12%)	
Moderate to severe	19 (58%)	7 (21%)	0.13	19 (58%)	2 (6%)	0.088
ERβ immunoreactivity in AEC						
Positive	22 (67%)	8 (24%)		25 (76%)	5 (15%)	
Negative	0 (0%)	3 (9%)	**0.010**	2 (6%)	1 (3%)	0.48
ERβ immunoreactivity in BEC						
Positive	22 (67%)	9 (27%)		26 (79%)	5 (15%)	
Negative	0 (0%)	2 (6%)	**0.039**	1 (3%)	1 (3%)	0.23

Data are presented as the number of cases and percentage in each group. *P*-values were evaluated by a cross-table using the chi-square test. *P*-values < 0.05 were considered significant, and are shown in bold. AEC, alveolar epithelial cell; BEC, bronchiolar epithelial cell.

[*] Degree of fibrosis was classified into two groups based on the modified method of Aschcroft et al. (1988), and Aschcroft grade 4 and 5 cases were categorized as mild, and, grade 6 and 7 cases as moderate to severe. In this study, no IP cases corresponded to Aschcroft grades 0–3.

[**] Degree of fibrosis was classified into two groups based on the method of Fulmer et al. (1979).

septa than male and ovariectomized mice (Antunes et al., 2010). Results of our present study also showed that the aromatase immunoreactivity in AEC among IP patients was significantly higher in patients with UIP pattern and significantly frequent in those with moderate to severe fibrosis (Table 3), which may indicate the profibrotic effects of estrogen locally produced by aromatase in AEC of human IP.

Estrogens are known to be involved in the process of skin wound healing (Ashcroft et al., 1999; Mills et al., 2005; Gilliver et al., 2007; Merlo et al., 2008). Ashcroft et al. (1999) demonstrated that estrogens accelerated skin wound healing with collagen synthesis, and Mills et al. (2005) suggested that the locally produced estrogens at wound sites contributed greatly to the process of skin wound healing. Idiopathic pulmonary fibrosis is considered in the spectrum of an abnormal process of wound healing provoked and propagated by continuous AEC injury (Selman and Pardo, 2006; Willis et al., 2006; Strieter and Mehrad, 2009), and activated AEC releases various profibrotic cytokines/growth factors, such as transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), endothelin-1 (ET-1), or epidermal growth factor (EGF) (Selman and Pardo, 2006; Willis et al., 2006; Strieter and Mehrad, 2009). Taken together with these previous reports and our present study, it is suggested that increased estradiol levels by aromatase in AEC play an important role in the pathology of IP compared to other inflammatory lung diseases. This study is descriptive, and further studies are required to clarify the significance of estrogens and aromatase in human IP.

Acknowledgments

We appreciate skillful technical assistance of Ms. Yayoi Takahashi (Department of Pathology, Tohoku University Hospital, Sendai, Japan), and Mr. Katsuhiko Ono (Department of Anatomic Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mce.2014.05.016.

References

Abe, K., Miki, Y., Ono, K., Mori, M., Kakinuma, H., Kou, Y., Kudo, N., Koguchi, M., Niikawa, H., Suzuki, S., 2010. Highly concordant coexpression of aromatase and estrogen receptor β in non-small cell lung cancer. Hum. Pathol. 41, 190–198.

American Thoracic Society and European Respiratory Society, 2001. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001, Am. J. Respir. Crit. Care Med. 2002, 165, 277–304.

Antunes, M.A., Abreu, S.C., Silva, A.L., Parra-Cuentas, E.R., Ab'Saber, A.M., Capelozzi, V.L., Ferreira, T.P., Martins, M.A., Silva, P.M., Rocco, P.R., 2010. Sex-specific lung remodeling and inflammation changes in experimental allergic asthma. J. Appl. Physiol. 109, 855–863.

Ashcroft, G.S., Greenwell-Wild, T., Horan, M.A., Wahl, S.M., Ferguson, M.W., 1999. Topical estrogen accelerates cutaneous wound healing in aged humans associated with an altered inflammatory response. Am. J. Pathol. 155, 1137–1146.

Ashcroft, T., Simpson, J.M., Timbrell, V., 1988. Simple method of estimating severity of pulmonary fibrosis on a numerical scale. J. Clin. Pathol. 41, 467–470.

Brass, D.M., McGee, S.P., Dunkel, M.K., Reilly, S.M., Tobolewski, J.M., Sabo-Attwood, T., Fattman, C.L., 2010. Gender influences the response to experimental silica-induced lung fibrosis in mice. Am. J. Physiol. Lung Cell. Mol. Physiol. 299, L664–L671.

Cakan, M., Aldemir, M., Topcuoglu, M., Altug, U., 2009. Role of testosterone/estradiol ratio in predicting the efficacy of tamoxifen citrate treatment in idiopathic oligoasthenoteratozoospermic men. Urol. Int. 83, 446–451.

Carey, M.A., Card, J.W., Voltz, J.W., Germolec, D.R., Korach, K.S., Zeldin, D.C., 2007. The impact of sex and sex hormones on lung physiology and disease. lessons from animal studies. Am. J. Physiol. Lung Cell. Mol. Physiol. 293, L272–L278.

Castagnetta, L.A., Agostara, B., Montalto, G., Polito, L., Campisi, I., Saetta, A., Itoh, T., Yu, B., Chen, S., Carruba, G., 2003. Local estrogen formation by nontumoral, cirrhotic, and malignant human liver tissues and cells. Cancer Res. 63, 5041–5045.

Couse, J.F., Lindzey, J., Grandien, K., Gustafsson, J.A., Korach, K.S., 1997. Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. Endocrinology 138, 4613–4621.

Demura, M., Demura, Y., Ameshima, S., Ishizaki, T., Sasaki, M., Miyamori, I., Yamagishi, M., Takeda, Y., Bulun, S.E., 2011. Changes in aromatase (CYP19) gene promoter usage in non-small cell lung cancer. Lung Cancer 73, 289–293.

Fulmer, J.D., Roberts, W.C., von Gal, E.R., Crystal, R.G., 1979. Morphologic–physiologic correlates of the severity of fibrosis and degree of cellularity in idiopathic pulmonary fibrosis. J. Clin. Invest. 63, 665–676.

Gharaee-Kermani, M., Hatano, K., Nozaki, Y., Phan, S.H., 2005. Gender-based differences in bleomycin-induced pulmonary fibrosis. Am. J. Pathol. 166, 1593–1606.

Gilliver, S., Ashworth, J., Ashcroft, G., 2007. The hormonal regulation of cutaneous wound healing. Clin. Dermatol. 25, 56–62.

Gustafsson, J.A., 1999. Estrogen receptor beta – a new dimension in estrogen mechanism of action. J. Endocrinol. 163, 379–383.

Ishibashi, H., Suzuki, T., Suzuki, S., Niikawa, H., Lu, L., Miki, Y., Moriya, T., Hayashi, S., Handa, M., Kondo, T., Sasano, H., 2005. Progesterone receptor in non-small cell lung cancer – a potent prognostic factor and possible target for endocrine therapy. Cancer Res. 65, 6450–6458.

Ishizuka, M., Hatori, M., Suzuki, T., Miki, Y., Darnel, A.D., Tazawa, C., Sawai, T., Uzuki, M., Tanaka, Y., Kokubun, S., Sasano, H., 2004. Sex steroid receptors in rheumatoid arthritis. Clin. Sci. (Lond.) 106, 293–300.

Ishunina, T.A., Fischer, D.F., Swaab, D.F., 2007. Estrogen receptor alpha and its splice variants in the hippocampus in aging and Alzheimer's disease. Neurobiol. Aging 28, 1670–1681.

King Jr., T.E., 2005. Clinical advances in the diagnosis and therapy of the interstitial lung diseases. Am. J. Respir. Crit. Care Med. 172, 268–279.

Mah, V., Marquez, D., Alavi, M., Maresh, EL., Zhang, L., Yoon, N., Horvath, S., Bagryanova, L., Fishbein, M.C., Chia, D., Pietras, R., Goodglick, L., 2011. Expression levels of estrogen receptor beta in conjunction with aromatase predict survival in non-small cell lung cancer. Lung Cancer.

Mah, V., Seligson, D.B., Li, A., Marquez, D.C., Wistuba, I.I., Elshimali, Y., Fishbein, M.C., Chia, D., Pietras, R.J., Goodglick, L., 2007. Aromatase expression predicts survival in women with early-stage non small cell lung cancer. Cancer Res. 67, 10484–10490.

Merlo, S., Frasca, G., Canonico, P.L., Sortino, M.A., 2008. Differential involvement of estrogen receptor alpha and estrogen receptor beta in the healing promoting effect of estrogen in human keratinocytes. J. Endocrinol. 200, 189–197.

Miki, Y., Suzuki, T., Tazawa, C., Yamaguchi, Y., Kitada, K., Honma, S., Moriya, T., Hirakawa, H., Evans, D.B., Hayashi, S., Ohuchi, N., Sasano, H., 2007. Aromatase localization in human breast cancer tissues. possible interactions between intratumoral stromal and parenchymal cells. Cancer Res. 67, 3945–3954.

Mills, S.J., Ashworth, J.J., Gilliver, S.C., Hardman, M.J., Ashcroft, G.S., 2005. The sex steroid precursor DHEA accelerates cutaneous wound healing via the estrogen receptors. J. Invest. Dermatol. 125, 1053–1062.

Morani, A., Warner, M., Gustafsson, J.A., 2008. Biological functions and clinical implications of oestrogen receptors alfa and beta in epithelial tissues. J. Intern. Med. 264, 128–142.

Murakami, H., Harada, N., Sasano, H., 2001. Aromatase in atherosclerotic lesions of human aorta. J. Steroid Biochem. Mol. Biol. 79, 67–74.

Nakamura, Y., Miki, Y., Suzuki, T., Nakata, T., Darnel, A.D., Moriya, T., Tazawa, C., Saito, H., Ishibashi, T., Takahashi, S., Yamada, S., Sasano, H., 2003. Steroid sulfatase and estrogen sulfotransferase in the atherosclerotic human aorta. Am. J. Pathol. 163, 1329–1339.

Niikawa, H., Suzuki, T., Miki, Y., Suzuki, S., Nagasaki, S., Akahira, J., Honma, S., Evans, D.B., Hayashi, Si., Kondo, T., Sasano, H., 2008. Intratumoral estrogens and estrogen receptors in human non-small cell lung carcinoma. Clin. Cancer Res. 14, 4417–4426.

Omoto, Y., Kobayashi, Y., Nishida, K., Tsuchiya, E., Eguchi, H., Nakagawa, K., Ishikawa, Y., Yamori, T., Iwase, H., Fujii, Y., Warner, M., Gustafsson, J.A., Hayashi, S.I., 2001. Expression, function, and clinical implications of the estrogen receptor beta in human lung cancers. Biochem. Biophys. Res. Commun. 285, 340–347.

Patrone, C., Cassel, T.N., Pettersson, K., Piao, Y.S., Cheng, G., Ciana, P., Maggi, A., Warner, M., Gustafsson, J.A., Nord, M., 2003. Regulation of postnatal lung development and homeostasis by estrogen receptor beta. Mol. Cell. Biol. 23, 8542–8552.

Prabhu, A., Xu, Q., Manigrasso, M.B., Biswas, M., Flynn, E., Iliescu, R., Lephart, E.D., Maric, C., 2010. Expression of aromatase, androgen and estrogen receptors in peripheral target tissues in diabetes. Steroids 75, 779–787.

Raghu, G., Collard, H.R., Egan, J.J., Martinez, F.J., Behr, J., Brown, K.K., Colby, T.V., Cordier, J.F., Flaherty, K.R., Lasky, J.A., Lynch, D.A., Ryu, J.H., Swigris, J.J., Wells, A.U., Ancochea, J., Bouros, D., Carvalho, C., Costabel, U., Ebina, M., Hansell, D.M., Johkoh, T., Kim, D.S., King Jr., T.E., Kondoh, Y., Myers, J., Muller, N.L., Nicholson, A.G., Richeldi, L., Selman, M., Dudden, R.F., Griss, B.S., Protzko, S.L., Schunemann, H.J., 2011. An official ATS/ERS/JRS/ALAT statement. idiopathic pulmonary fibrosis. Evidence-based guidelines for diagnosis and management. Am. J. Respir. Crit. Care Med. 183, 788–824.

Ryu, J.H., Daniels, C.E., Hartman, T.E., Yi, E.S., 2007. Diagnosis of interstitial lung diseases. Mayo Clin. Proc. 82, 976–986.

Schmidt, M., Weidler, C., Naumann, H., Anders, S., Scholmerich, J., Straub, R.H., 2005. Androgen conversion in osteoarthritis and rheumatoid arthritis synoviocytes–androstenedione and testosterone inhibit estrogen formation and favor production of more potent 5alpha-reduced androgens. Arthritis Res. Ther. 7, R938–R948.

Selman, M., Pardo, A., 2006. Role of epithelial cells in idiopathic pulmonary fibrosis. From innocent targets to serial killers. Proc. Am. Thorac. Soc. 3, 364–372.

Skliris, G.P., Leygue, E., Watson, P.H., Murphy, L.C., 2008. Estrogen receptor alpha negative breast cancer patients: estrogen receptor beta as a therapeutic target. J. Steroid Biochem. Mol. Biol. 109, 1–10.

Skliris, G.P., Parkes, A.T., Limer, J.L., Burdall, S.E., Carder, P.J., Speirs, V., 2002. Evaluation of seven oestrogen receptor beta antibodies for immunohistochemistry, western blotting, and flow cytometry in human breast tissue. J. Pathol. 197, 155–162.

Stabile, L.P., Davis, A.L., Gubish, C.T., Hopkins, T.M., Luketich, J.D., Christie, N., Finkelstein, S., Siegfried, J.M., 2002. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor alpha and beta and show biological responses to estrogen. Cancer Res. 62, 2141–2150.

Strieter, R.M., Mehrad, B., 2009. New mechanisms of pulmonary fibrosis. Chest 136, 1364–1370.

Suzuki, T., Miki, Y., Moriya, T., Akahira, J., Ishida, T., Hirakawa, H., Yamaguchi, Y., Hayashi, S., Sasano, H., 2007. 5Alpha-reductase type 1 and aromatase in breast carcinoma as regulators of in situ androgen production. Int. J. Cancer 120, 285–291.

Taylor, A.H., Al-Azzawi, F., 2000. Immunolocalisation of oestrogen receptor beta in human tissues. J. Mol. Endocrinol. 24, 145–155.

Vizoso, F.J., Rodriguez, M., Altadill, A., Gonzalez-Dieguez, M.L., Linares, A., Gonzalez, L.O., Junquera, S., Fresno-Forcelledo, F., Corte, M.D., Rodrigo, L., 2007. Liver expression of steroid hormones and Apolipoprotein D receptors in hepatocellular carcinoma. World J. Gastroenterol. 13, 3221–3227.

Voltz, J.W., Card, J.W., Carey, M.A., Degraff, L.M., Ferguson, C.D., Flake, G.P., Bonner, J.C., Korach, K.S., Zeldin, D.C., 2008. Male sex hormones exacerbate lung function impairment after bleomycin-induced pulmonary fibrosis. Am. J. Respir. Cell Mol. Biol. 39, 45–52.

Weinberg, O.K., Marquez-Garban, D.C., Fishbein, M.C., Goodglick, L., Garban, H.J., Dubinett, S.M., Pietras, R.J., 2005. Aromatase inhibitors in human lung cancer therapy. Cancer Res. 65, 11287–11291.

Willis, B.C., duBois, R.M., Borok, Z., 2006. Epithelial origin of myofibroblasts during fibrosis in the lung. Proc. Am. Thorac. Soc. 3, 377–382.