Expression pattern of estrogen receptor β and its correlation with multidrug resistance in non-small cell lung cancer

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The aim of this work was to determine the expression of the ERβ (estrogen receptor β) and multidrug resistance, namely MDR1 (P-glycoprotein, P-gp), in 152 samples of non-small cell lung cancer. The expression pattern of ERβ and MDR1 were assessed by the quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) and immunohistochemistry. We also analyzed the correlation between ERβ and MDR1 with clinical and pathological data. The co-expression pattern of ERβ and individual MDR1 proteins was assessed by correspondence analysis and chi-squared tests. In the present study, we found that patients with tumor stage I-II showed higher ERβ mRNA expression levels and decreased expression of ERβ protein with increasing tumor grade, which is opposite to MDR1 expression. In addition, an opposite co-expression pattern of ERβ and individual MDR1 proteins was also observed. In conclusion, the results can be used to better understand the expression control of MDR1 and may allow for the establishment of new cancer chemistry strategies that will control P-gp expression in NSCLC.

Key words: lung cancer, estrogen receptor β, MDR1, P-glycoprotein

Lung cancer is the cardinal cause of cancer-related deaths that threaten human health and life and its incidence and mortality are increasing worldwide [1]. Cancer statistics from 2018 estimated that lung cancer is expected to become the second most common cancer [2]. In China, lung cancer counts for 17.09% of all cancer cases with 24.35% mortality rate, and more, ranking it as the most lethal among all malignant tumor types [3]. Non-small cell lung cancer (NSCLC) includes lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD) and large cell carcinoma (LCLC). LUAD is one of the major histologic subtypes of lung cancer [4]. On the contrary to the LUSC, LUAD is more likely to occur in women and non-smokers [5]. Previous studies have shown that female patients with NSCLC have more favorable outcomes at different stages, suggesting the importance of gender as an independent prognostic factor for advanced NSCLC [6, 7]. The exact cause of this condition is unclear, but the potential role of sex hormones has been suggested as a possible explanation.

Etiology of NSCLC is a complex process of multi-gene participation and multiple stages. Risk factors for the initiation and development of NSCLC have not yet been studied clearly and the key molecular induced by these factors working in the progression of this disease are left unknown, causing both lack of the special explanations for the mechanisms of cancer and the biomarkers for the early diagnosis and prevention. Over the past few decades, increased exposure to environmental endocrine disruptors chemicals (EDCs) has been associated with the development of physical illnesses, such as high susceptibility to tumorigenesis or metastatic invasion potential [8]. Lesions triggered by exposures of environmental risk factors can activate oncogenes and inactivate suppressor genes, which end up with the formation of malignant tumor. Environmental estrogen is considered to be an endocrine disruptor compound that mimics the antagonist of estrogen in the body and interferes with the physiological functions of endogenous estrogen [9]. Therefore, environmental estrogen exposure has attracted more and more

attention. The discovery of estrogen receptor genes provided new clues about how estrogen signaling induces changes in cell function. Estrogen regulates a variety of biological processes, including cell proliferation, apoptosis, inflammation and metabolism, primarily through two classical estrogen receptor (ER) subtypes, including ER alpha (ERα) and ER beta (ERβ) [10–13]. Relative studies have shown that the expression of estrogen receptors is associated with the prognosis of NSCLC, but the roles of ERα and ERβ in tumor survival are complex [14–16]. Studies have shown that anti-estrogen therapy can reduce the risk of death in patients with NSCLC, suggesting that endogenous and exogenous estrogen may be associated with the etiology of NSCLC [17, 18].

Multidrug resistance (MDR) refers to the resistance of tumor cells to various chemotherapeutic drugs and remains one of the leading causes of cancer chemotherapy inefficiency [19]. It is generally described as unresponsiveness of an organ, tissue, cell or pathological condition treated with various drugs. P-glycoprotein (P-gp) is the most frequently tested indicator. It is one of the respiratory protection components and is found in various tissues with barrier function [20]. P-gp is known as multidrug resistance protein 1 (MDR1), which localizes on both, the bronchial and bronchiolar epithelium and the alveolar macrophage plasma membrane, and has been shown to act in a removal of environmental compounds from the lungs [21]. Xu et al. evaluated whether raloxifene hydrochloride (RAL) may sensitize MDR to chemotherapy in breast cancer, especially in estrogen receptor-negative (ER-) cases, only to find that RAL could significantly sensitize ER-related MDR breast tumors to paclitaxel [22].

In the present study, we hypothesized that estrogen levels are associated with tumor multidrug resistance, with the aim of exploring the co-expression relationship between ERβ and MDR in NSCLC.

Patients and methods

Patients and tissue samples. All procedures performed in studies involving human participants were in accordance with the Ethics Committee of Jiangsu Cancer Hospital (Nanjing, Jiangsu, China). Informed consent was obtained from all individual participants. Lung tumor tissues from 76 patients with NSCLC with relative clinical information in

this study were stored in RNAlater (Ambion; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and frozen at −80 °C. Adjacent non-tumor tissues were located >5 cm away from the edge of the tumor.

RNA extraction, cDNA synthesis and qRT-PCR. Expressions of ERβ, MDR1 gene and the normalization (housekeeping) gene UGT1A1 were assessed by the quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). Total RNA was extracted from tissue samples by the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and its purity was detected by NanoDrop 2000 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA (1 μg) was reverse transcribed into cDNA through a two-step reverse transcription reaction using the A214 reverse transcription system kit (GenStar, Beijing, China) according to the manufacturer's protocol. The primer sequences of mRNA for ERβ and MDR1 and housekeeping gene are listed in Table 1. All RNA primers were obtained from Generay Biotech Co., Ltd. (Shanghai, China). The PCR components comprised 1 μl cDNA, 5 μl Thunderbird SYBR qPCR mix, 0.3 μl PCR primers and 3.7 μl RNase-free water. Then, a two-step protocol (95 °C for 1 min, followed by 40 cycles of 95 °C for 15 sec, 60 °C for 30 sec and 72 °C for 30 sec) was undertaken. A cycle threshold (Ct) value is determined which indicates the number of (cycled) PCR cycles until the fluorescence reaches its threshold. Ct values were normalized by subtracting the Ct value of the housekeeping gene (UGT1A1) from the Ct value of the target gene (ΔCt). RNA results were calculated as 40−ΔCt values, which would correlate proportionally to the mRNA expression level of the target gene [23].

Immunohistochemistry. The tissues samples (5 μm thick sections) from another 76 patients with NSCLC obtained from the paraffin-embedded specimens were placed on glass slides. The embedded tumor tissues were routinely deparaffinized and endogenous peroxidase was quenched with 3% H_2O_2 in 1× PBS. Heating in sodium citrate buffer (pH 6.0) in a water bath at 95–100 °C for 30 min accomplished the epitope retrieval. Then, the sections were incubated for 1 hour with the primary antibodies with following dilutions: 1:50 of anti-ERβ (Dako; Agilent Technologies, Inc., Santa Clara, CA, USA), 1:100 of anti-P-gp (Dako; Agilent Technologies, Inc., Santa Clara, CA, USA). After that, the slides were washed twice with 1x PBS (5 min per wash) and incubated with the secondary antibody solution for 30 min at room temperature. Negative controls omitted primary antibodies as well as the use of isotype control antibodies. Visualization of ERβ and P-gp positive cells was done by using ABC staining system (Santa Cruz Biotechnology). Each slide was examined independently by two pathologists (from Jiangsu Cancer Hospital, Nanjing, Jiangsu, China) under a light microscope at ×400, ×200 and ×100 magnification, and 10 randomly selected fields of view were obtained. Cytoplasm or nucleus staining intensity and pattern that reflect the levels of ERβ, P-gp expressions were analyzed by using a composite score from 0 to 4+: 0, completely negative; 1+, faint positive for

Table 1. Sequences of the primers used in PCR analysis.

RNAs	Primer Sequence (5' to 3')
ERβ-F	TTCTCCTTCCTCCTACAACTG
ERβ-R	GATGTGATAACTGGCGATGG
MDR1-F	ATTTGACACCCTGGTTGGAG
MDR1-R	ACCACTGCTTCGCTTTCTGT
UGT1A1-F	AAGTGAACTCCCTGCTACCTT
UGT1A1-R	CCACTGGGATCAACAGTATCT

nuclear or cytosolic staining in <24% of cells; 2+, moderate positive staining covering between 25 to 49% of cells; 3+, positive staining covering between 50 to 74% of cells; 4+, strongly positive staining including >75% cells.

Statistical analysis. The statistical significance of qRT-PCR results was analyzed using GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Student's t-test (2-group t-test) and one-way ANOVA were carried out for the two-group comparison and gene expression of separate subgroups at the mRNA expression level, respectively. All data are expressed as the mean ± standard deviation.

To distinguish any differences in the distribution between high ERβ and P-gp from low expression groups, chi-squared tests or the Fisher's exact test for associations between ERβ and P-gp expression levels and clinicopathological data were performed [24]. The co-expression pattern of ERβ and P-gp were evaluated by the correspondence analysis and chi-squared tests. The threshold of P value was set as 0.05 to estimate the null hypothesis.

Results

Basic clinical and pathological data. A total of 152 NSCLC patients with relative clinical features (124 males and 28 females) were included (Table 2). The median age of the patients was 62.20 years (SD 8.85). 138 patients (90.8%) were diagnosed with adenocarcinoma and 14 (9.2%) with squamous cell carcinoma. Based on the eighth edition of the TNM classification of lung cancer from American Joint Committee on Cancer TNM staging system [25], among all the patients, there were 50 with tumor stage I (32.9%), 47 with tumor stage II (30.9%), 34 with tumor stage III (22.4%) and 21 with tumor stage IV (13.8%). Sixty-four (44.7%) of the patients had lymph node metastasis.

mRNAs expressions of ERβ and MDR1 in NSCLC patients. To assess mRNA expression of ERβ and MDR1, patients were randomized into two groups (76 patients per group, Figure 1). The expression of two target genes was assessed at the mRNA level by qRT-PCR. ERβ mRNA expression ranged between 24.37 to 41.12 with a median of 31.39. The expression range of MDR1 mRNA was 24.95–38.10, and the median was 31.09. In addition, we compared the mRNA expression of separate subgroups based on relative clinical features. Regarding the TNM staging classification, ERβ mRNA expression levels were significantly higher in patients with tumor stage I–II compared with advanced patients (Figure 2A) ($p < 0.05$). Other patient characteristics (G1, G2, G3) did not differ significantly in MDR1 and ERβ mRNA expression level (Figures 2B–2D).

Correlation between clinical features and expression of ERβ and P-gp. Immunohistochemistry was performed to investigate the expression pattern of ERβ and P-gp. Figure 3 demonstrates representative immunohistochemical staining of ERβ and P-gp in the nucleus and cytoplasm (×200 magnification). ERβ was mainly expressed in the nucleus and a

Table 2. Basic clinical characteristics of 152 NSCLC patients.

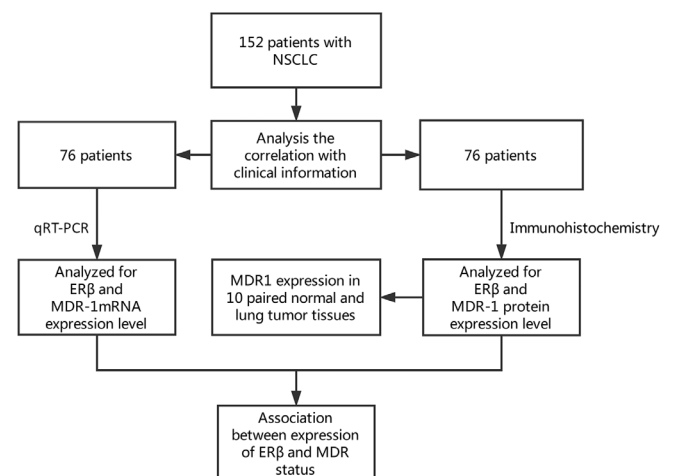| Variables	No. of cases (%)
Gender	
Female	28 (18.4%)
Male	124 (81.6%)
Age	
≤65 years	95 (62.5%)
>65 years	57 (37.5%)
TNM	
I	50 (32.9%)
II	47 (30.9%)
III	34 (22.4%)
IV	21 (13.8%)
T Stage	
T1	50 (32.9%)
T2	81 (53.3%)
T3	17 (11.2%)
T4	4 (2.6%)
Lymph node metastases	
N0	84 (55.3%)
N1	39 (25.7%)
N2	29 (19.0%)
Distant metastasis	
M0	131 (86.2%)
M1	21 (13.8%)
Histologic Stage	
G1	15 (9.9%)
G2	47 (30.9%)
G3	90 (59.2%)
Histology	
Adenocarcinoma	138 (90.8%)
Squamous cell carcinoma	14 (9.2%)

Figure 1. Flow chart of the analysis process.
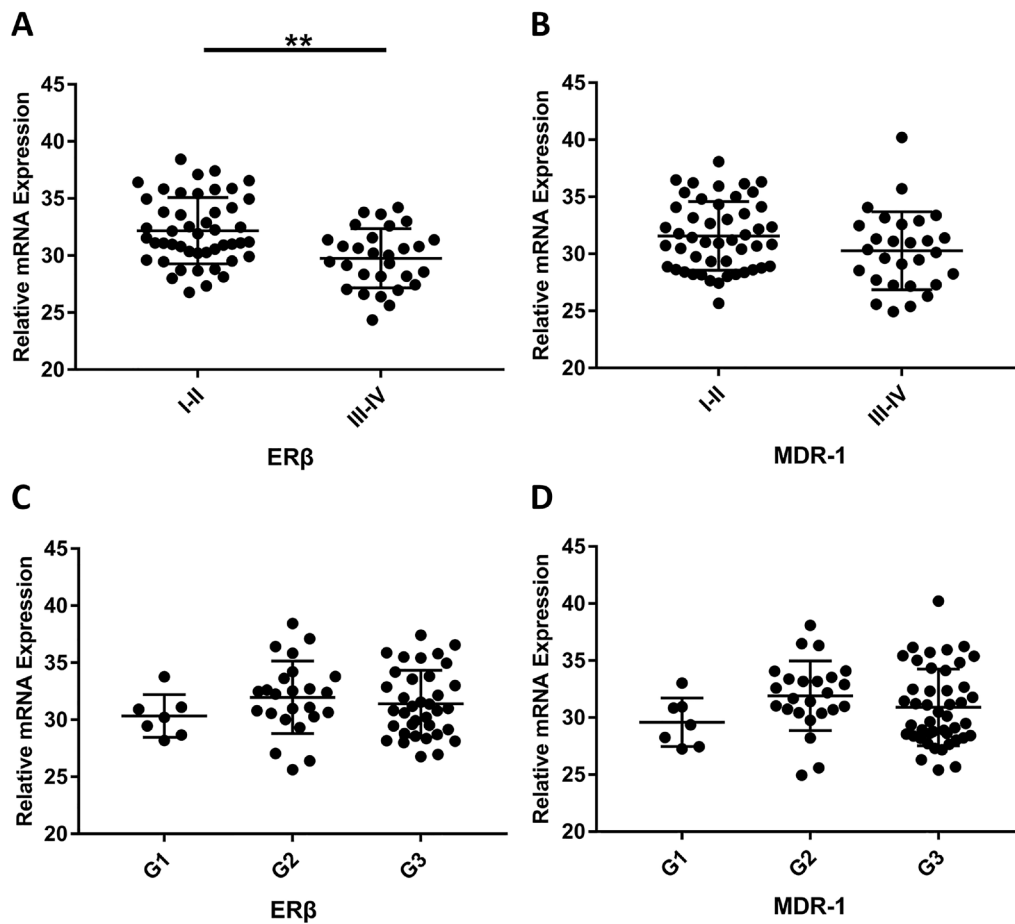
Figure 2. ERβ and MDR1 mRNA expression in NSCLC. A) Tumor tissue ERβ mRNA expression (I–II vs. III–IV); B) Tumor tissue MDR1 mRNA expression (I–II vs. III–IV); C) Tumor tissue ERβ mRNA expression (G1 vs. G2 vs. G3); D) Tumor tissue MDR1 mRNA expression (G1 vs. G2vs.S G3).

little part showed cytoplasmic localization, whereas P-gp staining was detected in the cytoplasm (Figure 3). A total of 61.8% (47/76) lung tumors were positive for nuclear ERβ expression, 89.5% (68/76) exhibited positive staining for cytoplasmic P-gp. Based on the expression status of ERβ (Negative or Positive), we analyzed the correlation between the two groups. There was no statistically significant difference in ERβ and P-gp expression associated with age, T Stage, lymph node metastases or distant metastasis status (Table 3). ERβ expression in patients with high tumor grade (G1, 87.5%) was higher than that in middle (G2, 60.9%) and low (G3, 60.0%) tumor grade (χ^2=10.421, p=0.004). In ERβ expression, 87.5% of grade 1 tumors were found to be positive. The expression of ERβ was only positive in 60.9% of grade 2 and 60% of grade 3 tumors. The result was consistent with previous study showing that a higher percentage of early tumors expressed ER receptors [26]. In addition, Pearson Chi-square analysis revealed that P-gp expression was significantly higher in G3 tumors (97.8%) compared with G1 (75.0%) and G2 (78.3%) (p<0.05) (Table 4).

To deeply investigate the correlation between ERβ and MDR1 protein expression level and clinicopathological data, we divided the patients into two groups according to the score of immunohistochemistry results (low expression group: 1+, 2+; high expression group: 3+, 4+). However, no statistically significant differences were found between the high or low ERβ and P-gp expression groups in tumor grade, age, tumor stage, lymph node metastases, distant metastasis and histologic stage (Table 3, Table 4).

Increased expression of P-gp in lung tumor tissues. To determine the role of P-gp expression in NSCLC, we performed immunohistochemistry on 10 pairs of samples consisting of both lung cancer tissues and adjacent normal lung tissue samples. In all samples, tumor tissues were MDR1-positive and three adjacent normal tissues were positive for P-gp staining. In lung tumor tissues we detected intracellular cytoplasmic localization of P-gp (Figure 4A). The IHC staining (composite score) showed a significantly increased expression of P-gp in NSCLC tissues compared to adjacent normal lung tissues (Figure 4B). Figure 5 shows the
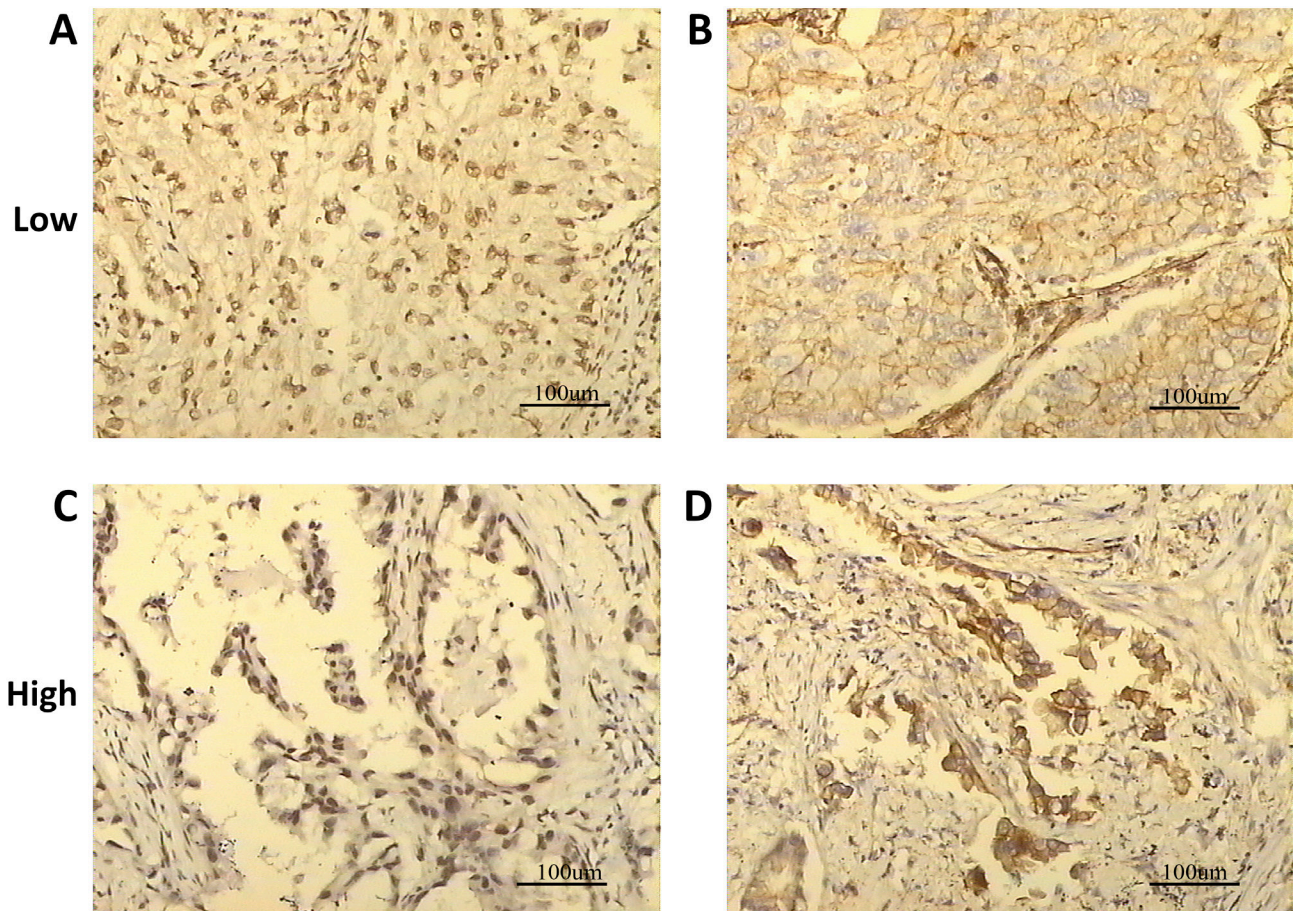
Figure 3. Immunohistochemical analysis of ERβ and MDR1 expression in NSCLC patients. Microscopic pictures at x 200 magnification, representing immunohistochemical staining of ERβ and P-gp expression. A) Tumor tissue ERβ low expression; B) Tumor tissue P-gp low expression; C) Tumor tissue ERβ high expression; D) Tumor tissue P-gp high expression.

different immunohistochemical expression level of P-gp in patients of NSCLC.

Correlation between ERβ and P-gp immunoreactivity. To further investigate the correlation between ERβ and P-gp, a correlation analysis was used to explore the association pattern between the ERβ and P-gp immunoreactivity levels (1+, 2+, 3+, 4+) [27]. The strength of association between each group could be interpreted as the distance separating two adjacent points. The smaller the distance, the greater the association between any particular group and other defined variables. Figure 6 showed that ERβ and P-gp were more closely related in the immunohistochemical high-level expression group (2+, 3+ and 4+).

Co-expression pattern of ERβ and individual P-gp were also assessed. The results indicated that a negative correlation between ERβ and P-gp was determined when P-gp low and P-gp high expression samples were compared. As shown in Table 3, in low P-gp expression group (n=26), highly concomitant ERβ expression (92.3%) was found in 24 tissue samples. However, in high P-gp expression group (n=17),

only 9 tissue samples were detected as high ERβ expression (52.9%). Chi-squared test results showed statistically significant differences between the two groups (p<0.01).

Discussion

The human respiratory system is often exposed to environmental pathogens and environmental endocrine disruptors chemicals. One of the most important processes at the cellular level is the pumping of chemicals from cells by means of transporting protein systems at cell membranes [20]. Multidrug resistance (MDR) is a major obstacle to the efficacy of cancer chemotherapy. The drug transporter P-glycoprotein (P-gp) protein is considered to be the contributor to P-gp-mediated multidrug resistance , encoded by the human multidrug resistance (MDR-1) gene [28]. The previous study has shown that ERα could increase expression of the MDR1 gene by directly activating gene transcription [29]. However, ERα and ERβ regulate gene expression in a classical and non-canonical way, inducing different biological responses

Table 3. Correlations between tumor clinicopathological parameters and ERβ expression.

| Variables	Total	Negative	Positive	χ^2	p-value	Total	Low (+ to ++)	High (+++ to ++++)	χ^2	p-value
ERβ expression										
Age										
≤ 65years	51	20	31	0.375	0.617	31	4	27	4.969	0.051
> 65years	25	8	17			17	5	12		
Tumor Stage										
I–II	47	19	29	0.946	0.457	28	5	23	0.035	1.000
III–IV	28	8	20			20	4	16		
T Stage										
T1	25	8	17	1.160	0.826	17	3	14	0.275	1.000
T2	39	14	25			25	5	20		
T3	10	5	5			5	1	4		
T4	2	1	1			1	0	1		
Lymph node metastases										
NO	44	14	30	1.021	0.336	31	4	27	1.964	0.247
YES	30	13	17			17	5	12		
Distant metastasis										
M0	62	26	36	3.752	0.068	36	7	29	0.046	1.000
M1	14	2	12			12	2	10		
Tumor grade										
G1	8	1	7	10.427	0.004	7	0	7	2.454	0.361
G2	23	9	14			31	6	25		
G3	45	18	27			10	3	7		
P-gp										
Negative	6	4	4	0.665	0.457					
Positive	68	24	44							
Low						26	2	24	8.925	0.004
High						17	8	9		
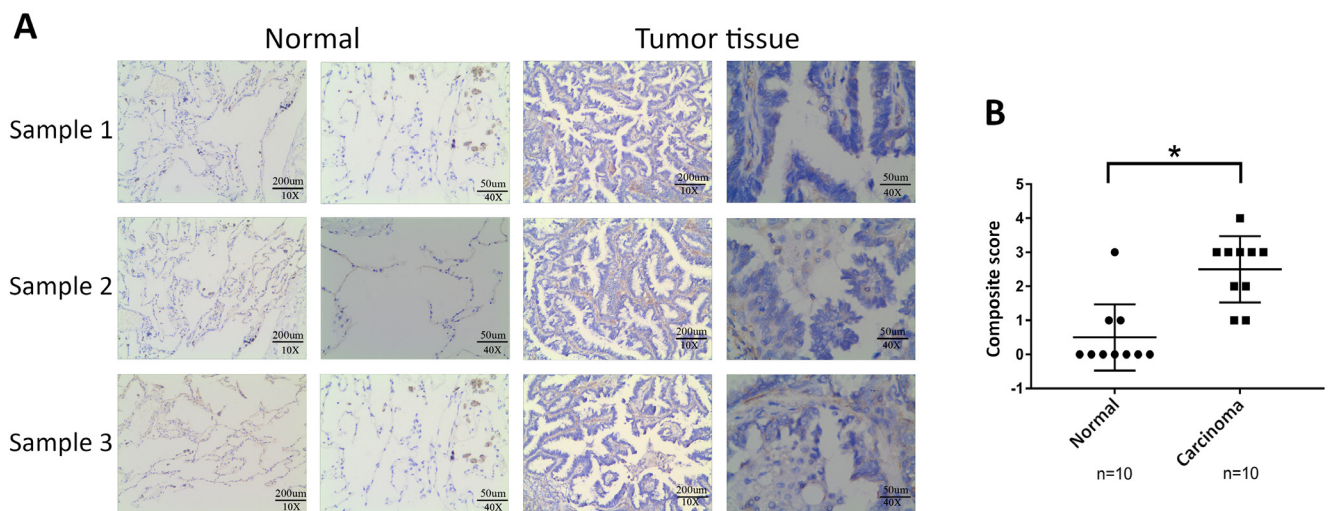
Figure 4. Expression of P-gp in lung cancer tissues and adjacent normal lung tissue samples. A) Immunohistochemical staining of tissues using P-gp antibody in both normal and lung tumor tissues. B) Graphical representation of the IHC composite score of each tissue sample. The composite score was calculated for each sample using the percentage of cells positive for P-gp staining. The graph was plotted using the composite score and p-values were calculated using 2-group t-test (p<0.05 considered as significant).

Table 4. Correlations between tumor clinicopathological parameters and P-gp expression.

| Variables						MDR1 expression				
	Total	Negative	Positive	χ^2	p-value	Total	Low (+ to ++)	High (+++ to ++++)	χ^2	p-value
Age										
≤65 years	51	7	44	1.685	0.259	44	26	18	0.154	0.799
>65 years	25	1	24			24	13	11		
Tumor Stage										
I–II	49	7	42	0.789	0.478	42	25	17	0.212	0.801
III–IV	27	2	25			26	14	12		
T Stage										
T1	25	3	22	0.338	1.000	22	9	13	4.844	0.199
T2	39	5	34			34	21	13		
T3	10	1	9			10	8	2		
T4	2	0	2			2	1	1		
Lymph node metastases										
NO	42	3	39	1.414	0.455	39	26	13	3.243	0.087
YES	34	5	29			29	13	16		
Distant metastasis										
M0	62	7	55	0.209	1.000	55	30	25	0.927	0.372
M1	14	1	13			13	9	4		
Tumor grade										
G1	8	2	6	8.415	0.018	6	5	1	2.224	0.330
G2	23	5	18			18	11	7		
G3	45	1	44			44	23	21		
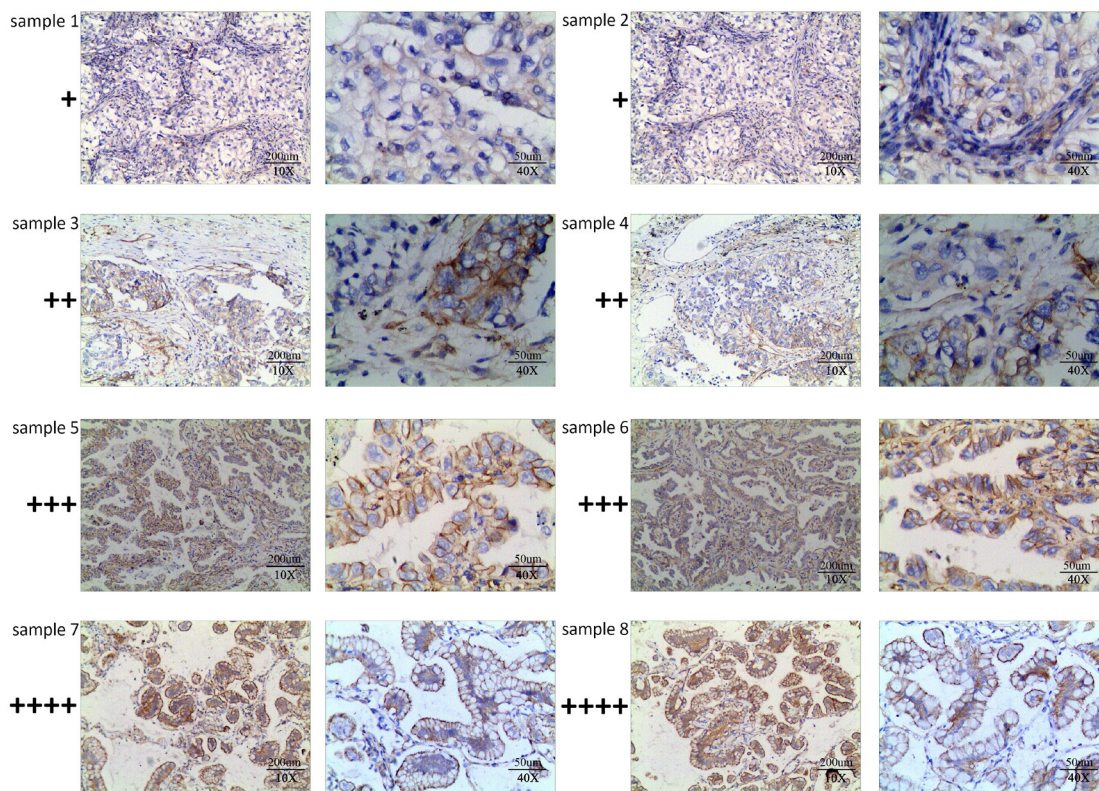
Figure 5. Representative immunohistochemical staining of P-gp in NSCLC tissue. All images were captured at magnification ×100 and ×400. P-gp expressions were analyzed by using a composite score from 0 to 4+: 0, completely negative; 1+, faint positive for nuclear or cytosolic staining in <24% of cells; 2+, moderate positive staining covering between 25 to 49% of cells; 3+, positive staining covering between 50 to 74% of cells; 4+, strongly positive staining including >75% cells.

[30]. Interestingly, Lindberg et al. have reported a "ying-yang" relationship between ERα and ERβ, and also found that ERβ is associated with antiproliferative effects [31]. The study of the link between the ERβ and MDR1 genes is drawing more attention, but it is still equivocal. Our specific aim was to explore the association of ERβ and MDR1 and to determine its correlation with clinicopathological parameters including age, TNM stage, T Stage, tumor grade, lymph node metastases or distant metastasis status. To delineate the expression patterns of ERβ and MDR1, we analyzed qRT-PCR and immunohistochemistry results in 152 patients with NSCLC.

We demonstrated that mRNA expression of ERβ was significantly associated with TNM stage, and patients with tumor stage I–II showed higher levels of ERβ mRNA expression (Figure 2). As the tumor stage progresses, the expression level of ERβ gradually decreases. Commensurate with previous reports, studies of breast cancer cell lines documented a decrease in ERβ expression during tumorigenesis [32, 33]. Unlike ERα, it is generally accepted that ERβ has a markedly different role and is considered to function as a tumor suppressor [34]. Mauro et al. determined that the lack of nuclear ERß was associated with poor prognosis in NSCLC patients [26]. Studies on gliomas also proved that declined expression of ERβ may be involved in the tumorigenesis, and its presence decreases with increased malignancy [35, 36].

According to reports, ERs are functional both at nuclear and extranuclear locations. We mainly detected ERβ expression at nuclear localization in NSCLC samples. ERβ and MDR1 (P-gp) expressions were detected in 47 (61.8%) and 68 (89.5%) out of 76 samples of NSCLC, respectively.
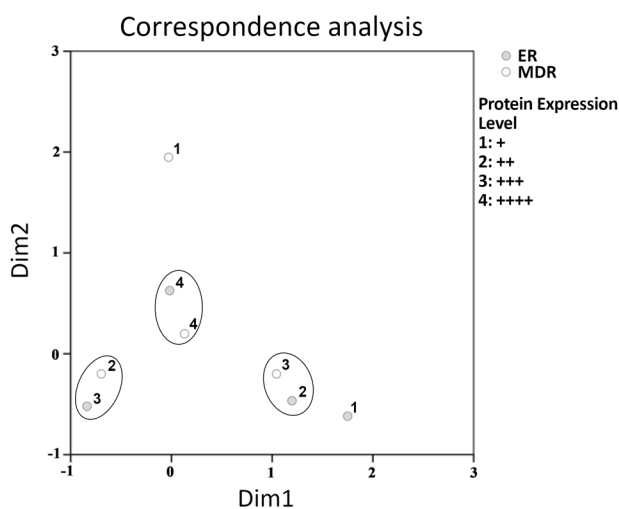

Figure 6. Correspondence analysis of the pattern of association between levels of ERβ and MDR1 immunoreactivity of NSCLC tissue samples. The strength of association between each group and level of immunoreactivity together can easily be interpreted in relation to the distance separating two adjacent points. The smaller the distance, the greater association there is between any specific groups.

More importantly, we found a negative correlation between ERβ protein expression and tumor grade. In grade 1 tumors, 7/8 (87.5%) tumors sample showed positive expression of ERβ. The positive expression rate of ERβ is progressively decreased in grade 2 and 3 tumors. Our results correlate well with previously studies in which ERs expression are correlated inversely with tumor grade and TNM stage in breast cancer [37]. Batistatou et al. reported that ERβ expression tends to decrease with increased histological malignancy of the tumor [38]. Although the therapeutic significance of ERβ signaling in lung cancer remains elusive, the current studies on the significance of expression and prognosis suggests that nuclear ERβ predicts better survival outcomes [26, 39–42].

Previous data suggest that P-gp expression in lung cancer is initially small but variable, and increases with disease progression and acquired resistance to cancer chemotherapy [19]. Similarly, we found that P-gp expression in NSCLC was positively correlated with tumor grade (Table 4), which is contrary to the expression trend of ERβ. In addition, studies have shown that P-gp expression is positively correlated with the invasion potential of tumor cells [43, 44]. The current results showed no significant differences in other clinical features between the ERβ and P-gp expression groups (Table 3, Table 4).

Although P-gp is associated with advanced cancer and drug failure, clinical validation trials for P-gp or other ABC (ATP-binding cassette) transporters are rarely established [45]. In addition, some studies showed that over-expression of P-gp is associated with MDR in different types of cancer, leading to poor patient prognosis [46–48]. We also demonstrated that increased expression of P-gp in lung tumor tissues (Figure 4) compared with adjacent non-tumor tissues, consistent with previous reports [49–51]. It is worth noting that some studies have correlated P-gp overexpression with negative survival outcomes, including treatment failure and relapse [52–54].

Researches have previously reported that estrogens inhibit breast cancer resistance protein (BCRP)-mediated drug resistance, which subsequently indicates that the estrogen sulfate conjugate is the physiological substrate of BCRP [55–58]. To date, there is evidence that ERα could directly regulate the expression of the MDR1 gene [29, 59]. However, only a few studies have used population-based cases and few have evaluated differences in ERβ expression by subject characteristics or association with MDR1 in NSCLC patients. Therefore, we evaluated the correlation between the levels of immunoreactivity of ERβ and P-gp based on the same patient. Correspondence analysis showed that ERβ and MDR1 were closely related in higher level of immunohistochemical expression (Figure 6). Next, we also explored the co-expression of ERβ and individual MDR1 protein (P-gp). The results showed a negative correlation between ERβ and MDR1 protein when MDR1 low and MDR1 high samples are compared (Table 3). Taken together, our analysis suggests that a decrease of ERβ in advanced NSCLC patients might lead to a gradual increase in MDR1 expression.

In conclusion, our data demonstrate the expression pattern of ERβ and MDR1 in NSCLC. We believe that the most important finding in this study is that as the tumor grade increases, the expression of ERβ gradually decreases which is contrary to the expression of MDR1, and the loss of ERβ can be explained by the progressive dedifferentiation of tumor cells. In addition, an opposite co-expression pattern of ERβ and individual MDR1 proteins was also observed. Despite some speculative explanations of this fact by the possible regulation of MDR1 expression through ERs. These results can be used to better understand the expression control of MDR1 and may allow for the establishment of new cancer chemistry strategies that will control P-gp expression in NSCLC, thereby increasing their sensitivity to MDR1-related anticancer agents.

The results of this study may have potential clinical significance, but it cannot be ignored that some limitations should be taken into consideration in this study. The influence of the single gene on the malignant biological behavior of NSCLC is affected by many kinds of uncertain factors, thus *in vivo* and *in vitro* experiments to explore the specific functions and molecular mechanism should be accomplished in the further study. Further evidence of the direct targeting regulatory relationship between ERβ and MDR1 is needed.

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