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The prognostic role of progesterone receptor expression in non-small cell lung cancer patients: Gender-related impacts and correlation with disease-specific survival

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ABSTRACT

Purpose: Progesterone has been shown to impact the development of hormone-sensitive cancers, such as breast and ovarian cancers. Emerging evidence has revealed a possible role of progesterone in the tumorigenesis of other cancers, including lung cancer. Herein, we aimed to elucidate the prevalence and prognostic significance of progesterone receptor (PR) expression in non-small cell lung cancer (NSCLC) tissue.
Experimental: Tumor tissue samples were collected from our patient cohort consisting of 335 NSCLC patients with stage I–IIIA disease. Tissue microarrays (TMAs) were constructed, and immunohistochemical (IHC) analyses were performed to evaluate the PR expression in the tumor epithelial and stromal compartments.
Results: In a univariate analysis, positive PR expression in the stromal tumor compartment ($P = 0.005$) was significantly and independently associated with a favorable outcome for both genders. Furthermore, positive PR expression in tumor epithelial cells ($P = 0.003$) correlated with a poor prognosis for female patients. In a multivariate analysis, positive PR expression in the tumor stroma ($P = 0.007$) was an independent prognostic factor for improved disease-specific survival (DSS). Positive PR expression in tumor epithelial cells emerged as an independent prognostic factor in female patients ($P = 0.001$) for poor DSS.
Conclusions: We show that PR expression in tumor-surrounding stromal cells is associated with improved DSS for both male and female patients. Additionally, we reveal that positive PR expression in tumor epithelial cells is an independent, unfavorable prognosticator for DSS in female patients, making PR expression a potential marker for prognostic stratification in NSCLC.

1. Introduction

The correlation between sex steroids and carcinogenesis has been studied for several decades, and it is well-established that estrogens and progesterone, the female sex steroids, contribute to the development of different cancer types, such as breast, ovarian and uterine cancer [1]. Research has also revealed a distinct association between estrogen and progesterone in the development of human non-small cell lung cancer (NSCLC) [2,3]. Their importance is supported by the observation that premenopausal women diagnosed with NSCLC have significantly reduced survival compared with postmenopausal women with decreased levels of endogenous sex steroids [4].

Estrogens serve several important roles in the human body and function as key regulators of normal sexual and reproductive physiology. Progesterone inhibits proliferation and promotes differentiation of the female reproductive tissues by binding to and activating the progesterone receptor (PR) [5]. This hormone acts as a transcription factor by binding directly to DNA, regulating

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the transcription of target genes [6]. PR consists of two isoforms, PRA and PRB, which are encoded by the same gene. PRA is primarily localized to the cell nucleus, where it functions as a ligand-dependent transcription factor [7,8] PRB is found in both the nucleus and cytoplasm, allowing PRB to shuttle between the two compartments and mediate both transcriptional changes and rapid cytoplasmic changes involving non-nuclear signaling pathways, such as the MAPK, PI3 K/Akt and c-Src pathways [7,9]. PRA and PRB likely act differently in cancer progression, and frequent alterations in the PRA/PRB ratio in breast cancer cases have been observed [8].

NSCLC constitutes 80–85% of all lung cancer cases and remains the leading cause of cancer-related deaths in the western world [10]. Despite marked progress in the field of cancer therapy in recent decades, the prognosis for NSCLC patients remains poor with a 5-year survival rate of 16% [10,11]. Investigational efforts are targeted to reveal new prognostic and predictive molecular markers to achieve more optimal therapy and improved overall survival for NSCLC patients.

Our research group has previously investigated the prognostic impact of several important biological markers related to angiogenesis [12], immunology [13,14] and hypoxia [15,16]. We aimed to investigate whether a correlation could be observed between PR and our previous markers and to determine the prognostic impact of PR expression in a representative NSCLC cohort of 335 NSCLC patients. We confirm previous findings presenting PR expression as an independent prognostic factor in non-small cell lung cancer. Furthermore our findings reveal important differences in PR expression in tumor epithelial cells versus tumor surrounding stromal cells and the correlation with DSS.

2. Experimental

2.1. Patients and clinical material

This retrospective study utilized primary tumor tissue from patients diagnosed with NSCLC stage I–IIIA; the tissue was surgically resected at the University Hospital of North Norway and Nordland Central Hospital between 1990 and 2004. Three hundred seventy-one patients were registered from the hospitals' databases. The following exclusion criteria were employed: (1) radiotherapy or chemotherapy prior to surgery, (2) other malignancy within 5 years before the NSCLC diagnosis and (3) inadequate paraffin-embedded tissue blocks. Thirty-six patients fell into these three categories (criteria 1: $n = 10$; criteria 2: $n = 13$; criteria 3: $n = 13$) and were excluded from the study. Adjuvant chemotherapy had not yet been introduced as a therapeutic option in Norway during this time span (1990–2004). In total, 335 patients with complete medical records and adequate paraffin-embedded tissue blocks were included in this study. The tumors were subtyped and histologically graded according to the World Health Organization (WHO) guidelines [17], and the patients were staged corresponding to the 7th edition of the UICC TNM classification [18]. The Regional Committee for Medical and Health Research Ethics, as well as the Norwegian Data Inspectorate, approved this study.

2.2. Microarray constructions

All of the lung cancer specimens were investigated thoroughly by two experienced pathologists (S.A.S. and K.A.S.). From the paraffin-embedded blocks, the most representative areas of (1) viable tumor epithelial tissue and (2) the tumor-surrounding stroma were selected. Tissue microarray (TMA) blocks were constructed using a tissue-array instrument (Beecher Instruments, Silver Springs, MD, USA) as previously described [12,19]. Two cores from the representative epithelial neoplastic area and two cores from the tumor-surrounding stromal areas were collected using a 0.6-mm-diameter stylet. The cores were then transferred to recipient blocks. Eight blocks were constructed to include all of the collected cores from the tissue samples. Normal lung tissue localized distant to the primary tumor was used as a control. Using a Micron microtome (HM355S), the cores were cut into multiple 4-μm sections, which were then stained with specific antibodies for immunohistochemical (IHC) analysis. The representative biomarker expression presented in the TMAs compared with that in regular sections showed a concordance >90–95% [20].

2.3. Immunohistochemistry

The Ventana Benchmark XT automated slide stainer (Ventana Medical Systems, Tucson, AZ, USA) was used for immunohistochemistry. Sections were rehydrated with ethanol after being deparaffinized with xylene. Antigen retrieval was performed by first placing the specimens in 0.01 M citrate buffer at pH 6.0 and then exposing the specimens to two treatments of repeated microwave heating for 10 min at 450 W. The DAKO EnVision + System-HRP (DAB) kit (DAKO, Glostrup, Denmark) was used for endogenous peroxidase blocking. The antibodies CONFIRM anti-PR (clone 1E2) antibody (Ventana Medical Systems), which recognizes the A and B isoforms of human PR, PDGFR-C (goat polyclonal, Gt15151, Neuromics, DLL-4 (rabbit polyclonal, Ab-7280, Abcam), p-BAD (goat polyclonal, Sc-7999, Santa Cruz) and CD20 (mouse monoclonal, L26, Ventana) were used. The antibodies were subjected to in-house validation by the manufacturer for immunohistochemical analysis on paraffin-embedded material. The supplier prepared the dilutions that were utilized. TMA staining of the antibodies and negative staining controls was performed in one single experiment. For the negative staining controls, the primary antibody was replaced with the primary antibody diluent. Additional details regarding the immunohistochemistry procedure have been previously described [21].

2.4. IHC scoring

The tissue cores were scored by light microscopy to determine the degree of nuclear PR expression. All of the anonymized samples were semiquantitatively and independently scored by two pathologists (S.A.S. and E.R.). The slides were re-examined in the case of disagreement, and a consensus was reached between the observers.

The dominant staining intensity in epithelial tumor cells and tumor surrounding stromal cells was scored as follows: 0 indicates negative, 1 indicates weak, 2 indicates intermediate, and 3 indicates strong. The staining density of stromal cells was scored as follows: 0 indicates 0%, 1 indicates 1–5%, 2 indicates 6–50%, and 3 indicates >50%, as described previously [12]. The mean scores were calculated for each case. Positive PR expression in both tumor cells and stromal cells was defined as expression $\geqslant$ the mean value of PR expression. In tumor cells, a positive score was defined as $\geqslant 1$, which was the closest even number to the mean score (0.82). No considerable differences were observed when the exact mean was used as a cutoff instead of 1. In stromal cells, the mean value of stromal cell intensity and density was calculated, and a sum $\geqslant 0.5$, the mean value, was considered to be positive expression. Staining in fibroblasts, fibrocytes and endothelial cells in the blood and lymph vessels were included in the stromal score. Previous scoring performed in our research group revealed an excessive staining of lymphocytes and plasma cells, and the staining of these cells was not decisive when evaluating stromal staining. Examples of positive and negative expression in the different compartments are shown in Fig. 1. The co-expression variable of PR in cancer and

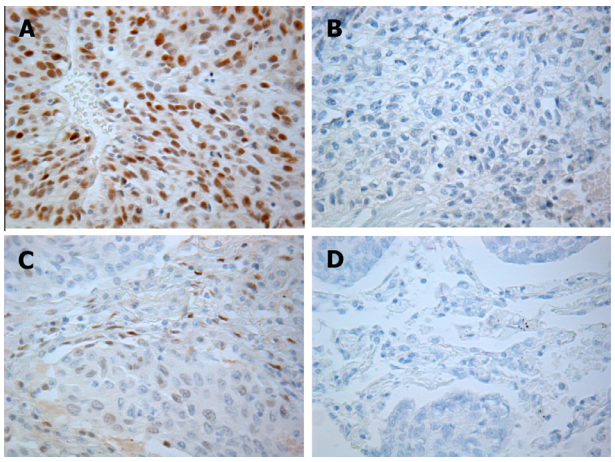

Fig. 1. Immunohistochemical analysis of PR expression in NSCLC. 400× magnification. Positive expression in (A) cancer cells and (C) stromal cells. Negative expression in (B) cancer cells and (D) stromal cells.

stromal cells was computed using the dichotomization low/low and other (high/low, low/high, high/high). High indicates positive PR expression; low indicates weak or negative PR expression. When evaluating the marker expression in each core, the observers were blinded to the patient outcome. There was a significant scoring agreement between the pathologists, with an intra-class correlation coefficient of 0.93 ($P < 0.001$).

2.5. Statistical analyses

The statistical analyses presented in this study were performed using the statistical package IBM SPSS, version 21 (SPSS Inc., Chicago, IL, USA).

Chi-squared and Fisher's exact test were used to examine the correlation among PR expression, different molecular markers and clinicopathological factors. The *r*-values represent Spearman's rank correlation coefficients. The Kaplan–Meier method was used to perform univariate analysis of the association between marker expression and disease-specific survival (DSS), which was the chosen endpoint. DSS was determined from the date of surgery until the time of lung cancer death. Statistical significance between the survival curves was assessed utilizing the log-rank test. The survival curves were terminated at 120 months due to fewer than 10% of patients at risk after this point. Variables that emerged as significant in the univariate analysis were included in a multivariate analysis, applying the Cox proportional hazards model. The data were run in a backward stepwise Cox regression with a probability for stepwise entry and a removal set at 0.05 and 0.10. The significance level was set at a *P*-value less than 0.05. The Wilcoxon non-parametrical rank test was used to ascertain differences in PR expression between the lung tumor and normal lung tissue in the same patient.

3. Results

3.1. Clinicopathological variables

Clinical, demographic and histopathological variables are presented in Table 1. Most of the patients were male (76%), and the median age was 67 years (range 28–85 years). Of the 335 NSCLC cases, 191 constituted squamous cell carcinomas (SCCs), 113 were adenocarcinomas (ACs), and 31 were large-cell carcinomas (LCCs). Fifty-nine patients (18%) were administered adjuvant radiotherapy due to nodal metastasis or non-radical surgical margins verified during surgery. The median follow-up of the survivors was 105 months (range 73–234 months). Among the patients, 96% were current or previous smokers.

3.2. Prevalence of PR in NSCLC cells

As expected, PR expression was primarily observed in the nucleus (Fig. 1). Positive PR expression in tumor cells ($\geqslant 1$) was detected in 115 (34.3%) of the 335 patients. Twenty-nine of these patients were women, and 86 were men. Considering the stromal expression of PR, 108 (32.2%) patients expressed a positive PR level ($\geqslant 0.5$). This patient group comprised 30 women and 78 men. Positive PR expression in tumor cells was detected in 64 (33.5%) cases of SCCs, 39 (34.5%) cases of ACs and 12 (38.7%) cases of LCCs. Fifty-four (28.3%) cases of SCCs, 40 (35.4%) cases of ACs and 14 (45.2%) cases of LCCs showed positive PR expression in stromal cells. A control sample group consisting of 42 cores of histologically normal lung tissue collected from our patient cohort, far from the site of the tumor, was used to compare PR expression in cancer versus non-neoplastic lung tissue. By applying the Wilcoxon non-parametrical rank test, we discovered that PR expression in normal lung tissue was equal to that in epithelial tumor tissue ($P = 0.025$). In the stromal compartment, however, we observed a significantly lower level of PR expression in the stromal cells ($P < 0.001$) surrounding the tumor tissue compared with non-neoplastic stromal cells.

3.3. Correlation of PR expression within the same gender and between genders

We found a significant correlation between PR expression in epithelial tumor cells and tumor-surrounding stromal cells ($r = 0.295$; $P < 0.001$). Following stratification, we observed a similar correlation between female patients and PR staining ($r = 0.304$; $P = 0.007$) and male patients ($r = 0.291$; $P < 0.001$). The correlation between PR expression (tumor epithelial and stromal cells) and clinicopathological prognosticators was weak or non-significant ($r < 0.2$). PR expression in tumor epithelial cells and correlation with clinicopathological prognosticators presented as follows: T-stage: $r = 0.013$; $P = 0{,}820$, N-stage: $r = 0.055$; $P = 0.326$, WHO performance status: $r = 0{,}106$, $P = 0.057$, histology: $r = 0.039$; $P = 0.483$, differentiation: $r = 0.042$, $P = 0.457$. The correlation between PR expression in tumor associated stromal cells and clinicopathological factors: T-stage: $r = -0.045$; $P = 0.414$, N-stage: $r = 0.023$; $P = 0.679$, WHO performance status: $r = -0.072$; $P = 0.196$, histology: $r = 0.114$; $P = 0.039$, differentiation: $r = 0.010$, $P = 0.854$. The remaining itemized clinicopathological variables are presented in Table 1.

3.4. Univariate analysis

The results from the univariate analyses regarding the clinical variables are shown in Table 1. WHO performance status ($P = 0.016$), histology ($P = 0.028$), differentiation ($P < 0.001$), surgical procedure ($P = 0.007$), pathological stage ($P < 0.001$), T-stage ($P < 0.001$), N-stage ($P < 0.001$) and vascular infiltration ($P = 0.001$) were significant prognostic variables. When stratified by gender, pathological stage ($P < 0.001$), N-stage ($P < 0.001$) and surgical margins ($P = 0.008$) were significant for women. However, for men, histology ($P = 0.043$), differentiation ($P < 0.001$), surgical procedure ($P = 0.011$), pathological stage ($P < 0.001$), T-stage ($P < 0.001$), N-stage ($P < 0.001$) and vascular infiltration ($P < 0.001$) were significant prognosticators.

PR expression in tumor epithelial and stromal cells and its influence on DSS are presented in Table 2 and Fig. 2. Positive PR expression in malignant epithelial cells was not a significant prognosticator. However, following gender stratification, positive PR

Table 1
Prognostic clinicopathologic variables as predictors of disease-specific survival in 335 NSCLC patients (univariate analyses; log-rank test).

Characteristic	Patients *N* (%)			Median survival (months)			5-year survival (%)			*P*-value		
	Combined	Female	Male	Combined	Female	Male	Combined	Female	Male	Combined	Female	Male
Age										0.42	0.49	0.56
⩽65 years	156 (47)	39 (48)	117 (46)	98	127	83	56	61	54			
⩾65 years	179 (53)	43 (52)	136 (54)	NR	NR	122	60	67	58			
Sex										0.22		
Female	82 (24)	82 (24)	253 (76)	190	190	98	64	62	56			
Male	253 (76)			98			56					
Smoking status										0.26	0.27	0.15
Never	15 (5)	6 (7)	9 (4)	19	21	18	43	50	38			
Previous	105 (31)	21 (26)	84 (33)	84	NR	NR	55	71	51			
Present	215 (64)	55 (67)	160 (63)	NR	NR	NR	60	63	60			
WHO performance status										**0.016**	0.053	0.096
ECOG 0	197 (59)	53 (65)	144 (57)	NR	NR	NR	63	67	62			
ECOG 1	120 (36)	27 (33)	93 (37)	64	127	51	52	63	49			
ECOG 2	18 (5)	2 (2)	16 (6)	25	19	36	33	0	40			
Histology										**0.028**	0.26	**0.043**
Squamous cell carcinoma	191 (57)	36 (44)	155 (61)	NR	NR	NR	66	77	63			
Adenocarcinoma*	113 (34)	38 (46)	75 (30)	54	69	43	46	56	41			
Large cell carcinoma	31 (9)	8 (10)	23 (9)	98	47	98	56	43	61			
Weight loss										0.76	0.61	0.97
<10%	303 (90)	74 (90)	229 (91)	190	190	84	58	65	56			
>10%	32 (10)	8 (10)	24 (9)	98	47	98	57	50	61			
Differentiation										**<0.001**	0.734	**<0.001**
Poor	138 (41)	28 (34)	110 (43)	47	NR	32	47	61	43			
Moderate	144 (43)	36 (44)	108 (43)	190	190	NR	65	63	66			
Well	53 (16)	18 (22)	35 (14)	NR	NR	NR	68	71	67			
Surgical procedure										**0.007**	0.493	**0.011**
Wedge + Lobectomy	243 (73)	64 (78)	179 (71)	190	190	NR	62	67	60			
Pneumonectomy	92 (27)	18 (22)	74 (29)	37	NR	30	47	50	47			
Pathological stage										**<0.001**	**<0.001**	**<0.001**
I	157 (47)	41 (50)	116 (46)	NR	190	NR	72	80	69			
II	136 (41)	29 (35)	107 (42)	62	NR	42	51	57	50			
IIIA	42 (12)	12 (15)	20 (12)	17	19	16	24	25	25			
T-status										**<0.001**	0.153	**<0.001**
1	85 (25)	23 (28)	62 (24)	190	190	NR	75	77	74			
2	188 (56)	42 (51)	146 (58)	84	NR	71	57	66	55			
3	62 (19)	17 (21)	45 (18)	25	190	19	37	41	36			
N-status										**<0.001**	**0.001**	**<0.001**
0	232 (69)	61 (75)	171 (67)	NR	190	NR	67	73	65			
1	76 (23)	11 (13)	65 (26)	35	47	29	43	40	44			
2	27 (8)	10 (12)	17 (7)	18	21	16	18	30	9			
Surgical margins										0.374	**0.008**	0.687
Free	307 (92)	74 (90)	233 (92)	190	190	84	59	67	56			
Not free	28 (8)	8 (10)	20 (8)	47	23	NR	48	38	53			
Vascular infiltration										**0.001**	0.352	**<0.001**
No	284 (85)	62 (76)	222 (88)	190	190	NR	62	68	60			
Yes	51 (15)	20 (24)	31 (12)	27	NR	25	33	52	24			

The significant variables are presented in Bold.
A *P*-value <0.05 was defined as statistically significant.
* 18 of these patients had bronchioalveolar carcinomas; NR, not reached.

expression in malignant epithelial cells ($P = 0.003$) in female patients was significantly associated with an unfavorable DSS (Fig. 2A). This finding was not observed in the male patient group (Fig. 2B). Positive stromal cell expression ($P = 0.005$) of PR was significantly associated with a favorable DSS for men and woman combined (Fig. 2C). When stratified by gender, positive PR expression in tumor-surrounding stromal cells was a significant prognosticator for a favorable DSS in male patients ($P = 0.041$), with a similar trend, although not significant, for women ($P = 0.060$).

Our data also revealed a significantly worse 5-year survival ($P < 0.001$) for women with a combination of positive PR expression in epithelial tumor cells and weak or negative PR expression in stromal cells (high/low) than in women with other combinations (low/low, high/high, or low/high) (Fig. 2D).

3.5. Multivariate analysis

The variables that were found to be significant in the univariate analyses were included in the multivariate analysis. The results are presented in Table 3. Positive PR expression in stromal cells was found to be significantly and independently associated with a better prognosis for both genders combined (HR: 1.74; 95% CI: 1.16–2.61; $P = 0.007$). For male patients alone (HR: 1.54; 95% CI: 0.96–2.46; $P = 0.072$), the marker did not reach statistical significance.

PR expression in tumor cells was found to be significant in the univariate analysis only for female patients. These results were also significant in the multivariate analysis (HR: 3.46; 95% CI: 1.63–7.34; $P = 0.001$).

3.6. Correlations between PR expression and other molecular markers

Several correlations between PR and different biological markers were observed. The expression of platelet-derived growth factor-C (PDGF-C), which is important in angiogenesis [22] and connective tissue function, growth and survival [23], in stromal cells was negatively correlated with PR expression in epithelial cells ($r = -0.237$; $P < 0.001$). DLL4 is a vascular-specific Notch ligand that is essential in embryonic vascular arteriogenesis and development [24]. Tumor cells expressing DLL4 and PR-expressing tumor cells correlated positively ($r = 0.243$; $P < 0.001$). A significant inverse

Table 2
PR expression in cancer cells and tumor stromal cells as a predictor of DSS in 335 NSCLC patients (univariate analysis; log rank test).

Characteristics	Patients, *N* (%)			Median survival (months)			5-year survival (%)			*P*-value		
	Combined	Female	Male	Combined	Female	Male	Combined	Female	Male	Combined	Female	Male
PR												
Cancer cells										0.356	**0.003**	0.673
Positive	115 (34)	29 (35)	86 (34)	NR	47	NR	54	43	58			
Weak or negative	207 (62)	48 (59)	159 (63)	190	190	75	60	76	55			
Missing	13 (4)	5 (6)	8 (3)									
Stromal cells										**0.005**	0.060	**0.041**
Positive	108 (32)	30 (37)	78 (31)	NR	NR	NR	69	76	67			
Weak or negative	220 (66)	49 (60)	171 (67)	71	127	71	53	56	52			
Missing	7 (2)	3 (3)	4 (2)									
Cancer cells + stromal cells[*]										**0.010**	**<0.001**	0.213
Low/high	45 (13)	12 (15)	33 (13)	NR	NR	NR	79	100	71			
Low/low	162 (49)	36 (44)	126 (50)	74	190	62	55	68	51			
High/high	58 (17)	16 (19)	42 (17)	NR	NR	NR	61	60	62			
High/low	57 (17)	13 (16)	44 (17)	37	23	NR	46	20	54			
Missing	13 (4)	5 (6)	8 (3)									

NR, not reached.
The significant variables are presented in Bold.
[*] Low/high = negative or weak epithelial PR expression, positive stromal PR expression, high/low = positive epithelial PR expression, weak or negative stromal PR expression.

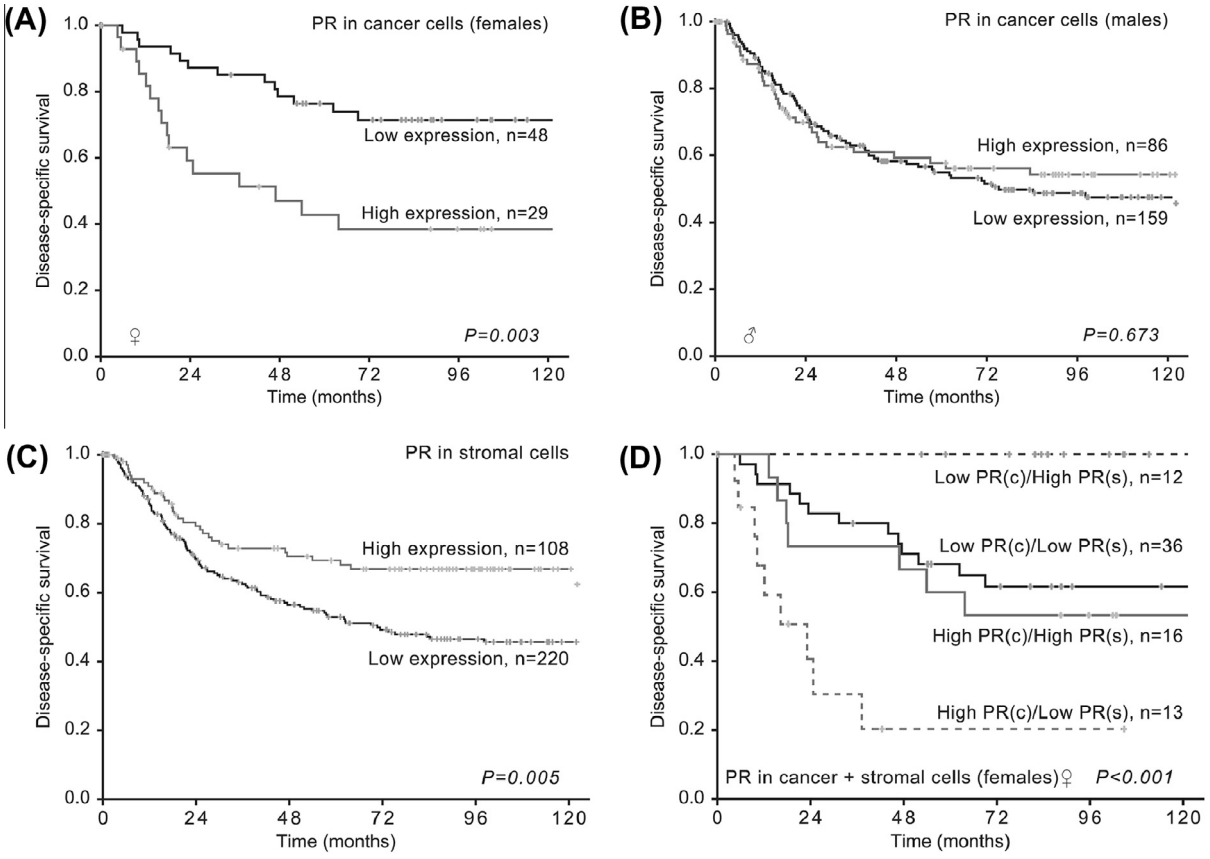

Fig. 2. Kaplan–Meier curves of disease-specific survival according to PR expression (A) in cancer cells from females and (B) males, (C) in stromal cells, and (D) in cancer and stromal cells from females. (c) = cancer cells, (s) = stromal cells.

correlation was observed between the pro-apoptotic marker BAD in tumor cells and PR-expressing tumor cells ($r = -0.206$; $P < 0.001$). This negative correlation was strengthened ($r = -0.326$; $P = 0.004$) when examining the female patients separately. Investigation of co-expression levels of these markers with PR, showed no significant correlation with survival compared with the effect seen with PR alone. We observed no correlation between PR expression in tumor epithelial cells and their expression of immune marker CD-20 ($r = -0.088$; $P = 0.117$). The expression of CD-20 did not correlate with stromal PR expression either ($r = -0.25$; $P = 0.655$).

4. Discussion

This is the first study to investigate the various effects of PR expression in different cellular compartments in an unselective cohort of NSCLC patients. In the univariate analyses, positive PR expression in epithelial tumor cells in female patients was significantly and independently associated with a poor prognosis.

In tumor-surrounding stromal cells, we observed positive prognostic significance of PR expression in both genders. Additionally, we discovered several potentially interesting correlations between

Table 3
Results of the Cox regression analyses (backward stepwise model) for clinicopathological variables and PR expression in tumor epithelial and stromal cells.

Factor	All patients, *N* = 335			Female patients, *N* = 82			Male patients, *N* = 253		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Tumor status			**<.001**	NE	NE	NE			**0.001**
T1	1.00						1.00		
T2	1.59	(1.00–2.53)	0.052				1.78	(1.00–3.15)	**0.049**
T3	3.05	(1.79–5.22)	**<.001**				3.48	(1.79–6.76)	**<.001**
Lymph node status			**<.001**			**0.002**			**0.001**
N0	1.00			1.00			1.00		
N1	1.98	(1.31–2.99)	**0.001**	3.93	(1.49–10.33)	**0.006**	1.54	(0.97–2.44)	**0.049**
N2	3.15	(1.86–5.34)	**<.001**	4.02	(1.62–9.99)	**0.003**	3.59	(1.81–7.13)	**0.001**
Differentiation			**<.001**	NE	NE	NE			**0.021**
Well	1.00						1.00		
Moderate	1.09	(0.59–1.99)	0.788				1.14	(0.54–2.38)	0.731
Poor	1.94	(1.08–3.48)	**0.026**				2.06	(1.01–4.21)	**0.047**
ECOG performance status			0.**025**	NE	NE	NE	NE	NE	NE
Normal	1.00								
Slightly reduced	1.61	(1.11–2.33)	**0.011**						
In bed >50%	1.77	(0.77.4.00)	0.168						
Vascular infiltration			**0.008**	NE	NE	NE			
No	1.00						1.00		
Yes	1.92	(1.22–3.04)	**0.005**				2.89	(1.69–4.87)	**<.001**
Histology			**0.001**	NE	NE	NE			**0.006**
Squamous carcinoma	1.00						1.00		
Adenocarcinoma	1.98	(1.36–2.89)	**<.001**				1.90	(1.23–2.93)	**0.004**
Large cell carcinoma	0.99	(0.52–1.91)	0.986				0.83	(0.40–1.71)	0.606
Surgical margins	NE	NE	NE				NE	NE	NE
Free				1.00					
Not free				2.61	(1.05–6.52)	**0.040**			
PR stromal cells				NE	NE	NE			
Positive	1.74	(1.16–2.61)	**0.007**				1.54	(0.96–2.46)	0.072
Weak or negative	1.00						1.00		
PR cancer cells	NE	NE	NE				NE	NE	NE
Positive				1.00					
Weak or negative				3.46	(1.63–7.34)	**0.001**			

The significant variables are presented in Bold.
HR, hazard ratio; CI, confidence interval; NE, not entered (not significant in univariate analysis); NS, not significant.
Significant clinicopathological factors and molecular markers from the univariate analysis were included in this analysis.

the expression levels of PR and proteins involved in angiogenesis (PDGF-C), apoptosis (BAD) and Notch signaling (DLL4).

Various studies have reported conflicting results regarding PR expression and its implications in NSCLC cells. In 2005, Ishibashi et al. [3] reported that PR expression was a strong positive prognostic factor in NSCLC. The authors found a high prevalence of positive PR cases (47%) in their cohort, which was slightly larger than in our epithelial tumor and stromal cells (32–34%). We observed decreased PR expression in the tumor-surrounding stromal tissues compared with the PR expression level in non-cancerous stromal tissue. Previous reports from Stabile et al. [25] and Marquez-Garban et al. [26] also demonstrated a lower expression of PR transcripts in non-cancerous tissue compared with malignant epithelia, however neither of the reports distinguished expression in tumor epithelia vs tumor stroma. Reports regarding PR expression and the localization of receptors in neoplastic tissue conflict with studies reporting high expression [25–27] and low or no expression [28,29]. These conflicting results may be explained by several factors, including the interpretation and scoring of the stained tissue, specificity and sensitivity of antibodies and differences between the patient cohorts, contributing to the lack of standardization.

Our observations of PR as a positive prognosticator when expressed in tumor-surrounding stroma have been confirmed by previous publications that demonstrated the association of low PR expression with poor clinical outcome in NSCLC [3,29]. Bogina et al. [30] claimed that loss of PR expression was associated with a poor prognosis. Other studies have revealed no correlation between PR and patient outcome [11,27].

None of the aforementioned studies evaluated the prognostic impact of PR expression according to compartment localization (stroma versus tumor). Our results imply that stromal PR expression may play a protective role in tumor development, whereas epithelial PR expression promotes tumor growth. We observed no correlation between PR expression in tumor and stromal compartment and the expression of immune cells, suggesting that the stromal cells are working independently without help from the immune system to fight the tumor. We propose this hypothesis as an explanation for our observation that females with weak or negative tumor epithelial/positive stromal PR expression have a significantly favorable DSS. Consequently, PR expression in different cellular compartments has discrepant effects on tumorigenesis.

Bilateral and continuous molecular crosstalk occurs between cells constituting the stromal compartment and epithelial cells; this communication is mediated by secreted molecules or by direct cell–cell contact [32]. Hence, minor changes in one compartment may cause massive alterations in the system. This crosstalk may also be conducted through reciprocal signals that regulate the expression or activity of hormone receptors. This crosstalk has previously been reported for prostate cancer, in which cancer-initiating fibroblast growth factor signals, originating from the stroma, caused an increase in androgen receptor expression in prostate epithelia [33]. Recently, similar findings have been described for endometrial cancer [34]. Oncogenic alterations in the endometrial tumor epithelia caused decreased levels of PR in tumor stroma, resulting in progesterone resistance.

Our data indicate that positive PR expression in tumor cells represents a negative prognostic factor in female patients. There are different explanations for progesterone's negative prognostic impact in cancer cells involving the activation of tissue factors such as EGF and VEGF. Marquez-Garban et al. [26] demonstrated that combined treatment with progesterone and estrogen stimulate VEGF secretion by NSCLC cells as previously described in breast

cancer cells [35]. Tumor cell VEGF production is essential in malignant development through the induction and upregulation of angiogenesis [36]. Hyder et al. [37] stated that progestin exerts its tumorigenic effects in the breast by enhancing angiogenesis. Our data demonstrate a correlation between PR expression and the angiogenic marker PDGF-C. Although previous publications have described the role of estrogen in regulating angiogenic markers [38,39], limited information is available regarding progesterone's role in regulating tumor-associated angiogenesis in human lung cancer [26].

The diverse prognostic impacts of PR expression in different cancer types [2,3,26,34,37], may be explained by the overall balance between pro- and anti-tumorigenesis genes that are activated upon progesterone signaling in a specific tumor. It is unknown how PR signaling is conducted in NSCLC cells. Older men and women have the highest risk for developing lung cancer. These individuals have very low concentrations of endogenous progesterone. Here, PR signaling may be ligand-independent, as in the breast, through kinases located in the lung [3,7,25]. Because *in situ* synthesis of progesterone in NSCLC specimens has been demonstrated, this process may also be involved in tumorigenesis [3].

To our knowledge, our study is the first to demonstrate a significant inverse correlation between PR expression and the BCL-associated death protein (BAD) in NSCLC. BAD is a pro-apoptotic member of the BCL-2 family. A previous publication reported that the progesterone-dependent downregulation of BCL-2 in breast cancer cells [40] may allow damaged cells with mutated DNA to avoid apoptosis, resulting in neoplastic formation.

Janzen et al. [34] concluded that stromal PR expression may emerge as a reliable biomarker to predict the response to hormonal therapy in endometrial cancer. *In vitro* and *in vivo* studies have shown that progesterone administration inhibits the growth of PR-positive NSCLC cell lines [3]. Our results show that female patients with positive PR expression in epithelial cells had a poor prognosis. It will be important to elucidate the different impacts of PR expression in tumor epithelial tissue vs. tumor-associated stromal tissue and to determine whether PR expression is a biomarker for hormonal therapy responsiveness in this patient group.

Our study is strengthened by the use of a reliable antibody that is frequently used in the clinical detection of PR expressing breast cancer cells. The supplier (Ventana Medical Systems) has performed western blot analyses, thus ensuring the specificity of the antibody. Our results present the different impacts of PR expression in both malignant epithelial and stromal cells and provides a more adequate overview of the impact of PR expression in tumorigenesis. The size of our patient cohort (335) may represent a potential weakness of our study, and our results will have to be validated in a larger patient group. It is also pivotal to establish a scoring template to standardize the immunohistochemical interpretation of PR expression.

5. Conclusion

Herein, we confirm that PR is an independent prognostic marker in NSCLC. Moreover, we highlight the importance of female sex steroids in the development of NSCLC. Our results substantiate the diverging impact of PR expression in different cellular compartments and genders, emphasizing the importance of future studies to elucidate PR expression in NSCLC.

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7. Disclosure statement

The authors have nothing to disclose.

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