

## Estrogen and Progesterone Receptors in Non Small Cell Lung Cancer Patients

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**The role of sex hormones in the pathogenesis of lung cancer is still unknown. There are conflicting results regarding immunohistochemical detection of the estrogen and progesterone receptors expression in non small cell lung cancer. To clarify these discrepancies 32 samples of lung carcinoma tissues obtained by lobectomy or pneumonectomy were studied. Two monoclonal antibodies (6F11 and ID5) for estrogen receptor detection and one (1A6) for progesterone receptor detection were used.**

**Eighteen adenocarcinoma and 14 squamous cell carcinoma cases were investigated. There were 11 women and 7 men with adenocarcinoma and 4 women and 10 men with squamous cell carcinoma.**

**Weak (+1) nuclear estrogen hormone receptor expression was detected in only one specimen of a woman with adenocarcinoma and in one specimen of a man with squamous cancer. None of the 32 blocks of paraffin embedded specimens expressed progesterone receptor.**

**The positive estrogen and progesterone receptors expression in cancer tissue is an important argument against the pulmonary origin of the unknown primary tumor. (Ann Thorac Cardiovasc Surg 2002; 8: 69–73)**

**Key words:** lung cancer, estrogen receptor, progesterone receptor, adenocarcinoma, squamous cell carcinoma

### Introduction

Although smoking is the major cause of lung cancer, it has been suggested that other factors act as carcinogens, particularly in nonsmoking women.<sup>1–4)</sup>

Epidemiology of lung cancer differs between sexes and it has been underlined that it might be partially explained by different sex hormone levels in women and men. There are some epidemiological data indicating that gender is a significant, independent prognostic factor in lung cancer. Particularly women with adenocarcinoma had a better prognosis than the patients with other histological types of cancer and also men with adenocarcinoma.<sup>1,2,5)</sup>

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Human estrogen receptor (ER) is a hormone activated nuclear transcription factor that is a regulator of growth and differentiation of responsive cells.<sup>6,7)</sup> Steroid hormone receptors are revealed in normal tissues: skin, bone, adrenal gland and thyroid but also in malignancies such as breast, endometrial, ovarian, melanoma, osteosarcoma, meningioma, colorectal, hepatocellular, renal cell, pancreatic, and others.<sup>8–12)</sup> The full understanding of the significance of ER in the development of reproductive malignancies and other cancers remains unknown. Positive expression of ER is a good predictor of the survival for breast and endometrial cancer but determines poor prognosis for gastric carcinoma.<sup>13)</sup> Clinical trials have shown that adjuvant antiestrogen therapy of postmenopausal women with estrogen receptor positive breast cancer is correlated with better prognosis.<sup>5,14)</sup>

During embryogenesis sex hormones influence the development of lung tissue, but during adulthood, the lung is not a target organ for sex hormones. There is evidence that estrogen can influence the growth of lung carcino-

mas.<sup>7,15)</sup> The data on expression and role of steroid hormone receptors as transcription factors in initiating and maintaining the growth of lung cancer is limited and far from conclusive.<sup>8,15-21)</sup>

From a practical point of view it is important to differentiate the origin of tumor tissue on the basis of immunostaining, particularly breast from lung cancer. Therefore we attempted to determine expression of estrogen and progesterone receptors by routinely using two different types of monoclonal antibodies.

## Materials and Methods

Material for immunohistochemical analysis was obtained by surgery (lobectomy or pneumonectomy) from 32 lung cancer patients treated in the National Tuberculosis and Chest Diseases Research Institute in 1998 and 1999. The mean age of patients was 62 years with a range from 42 to 78 years. There were 15 women and 17 men. Among them adenocarcinoma was diagnosed in 18 cases (11 women and 7 men). Squamous cell carcinoma was revealed in 14 cases (4 women and 10 men). All original glass slides from each case were reviewed microscopically, assessed separately by two authors and tumors were classified according to established WHO criteria (1999).<sup>22)</sup>

Immunohistochemical detection of sex steroid receptors was performed by the avidin-biotin-peroxidase complex (ABC-DAKO A/S, Glostrup, Denmark) system using two commercially available monoclonal antibodies. Mouse monoclonal antibodies-clone 6F11 (Novocastra, Newcastle, UK), and clone ID5 (DAKO A/S, Glostrup, Denmark) were used for estrogen receptor protein detection in paraffin embedded sections from formalin fixed tumors.

Progesterone receptor was detected by the use of mouse monoclonal antibody clone 1A6 (Novocastra, Newcastle, UK). Heat-induced (microwave) antigen retrieval in citrate buffer was used in all assays.

Diaminobenzidine (DAB) (DAKO A/S, Glostrup, Denmark) was used as the chromogen at a concentration of 0, 5 mg/ml in TRIS buffer (pH 7.6) in the presence of hydrogen peroxide (25  $\mu$ ml of 3% H<sub>2</sub>O<sub>2</sub> / 5 ml DAB). The following dilution's antibodies were used: DAKO-ER 1D5-1:50, Novocastra-ER 6F11-1:60, Novocastra PGR 1A6-1:40.

Slides were reviewed in a blinded fashion by two authors (RL, DG). Tumors were assessed as positive for the presence of receptors when both authors observed over 10% of nuclear immunoreactivity in malignant cells.

(Figs. 1, 2) The results were reported as weak positive when 10-50% cells, and strong positive when cell staining in over 50% was cell staining in was observed. The brown colour marks the presence of the receptor in the nucleus of cells. The receptor negative cells are blue because of counterstaining of the tissue with haematoxylin-eosin.

Negative (normal prostate tissue) and positive (normal breast and endometrial tissue) tissue controls were prepared for each set of slides. (Figs. 3, 4, 5)

## Results

Only in two specimens was estrogen receptor immunoreactivity detected. One was revealed by monoclonal antibody, clone 6F11 in a 59 year-old woman with adenocarcinoma and in a 52 year-old man with squamous cell carcinoma by the use of monoclonal antibodies, clone ID5. (Fig. 1) In both cases over 10% of cells expressed the weak positive nuclear staining.

No evidence of progesterone receptor immunoreactivity was present in any of the tumors (Table 1).

## Discussion

Sex steroid receptors detection and function are better known from studies of breast cancer cells.<sup>9,12,23)</sup> It has been recently shown that breast cancer cells contain the high levels of ER variants lacking multiple exons. The differences in amounts and molecular structures of ER variants between normal and breast cancer tissue imply that ER-dependent estrogen responsiveness might change during tumor development.<sup>23,24)</sup>

The role of sex steroid receptors in the development and progression of lung cancer is not known. Only limited data regarding detection of estrogen and progesterone receptors in lung cancer tumors and cell lines are available. By the use of a biochemical method, ERs expression varies between 16, 6% and 40%.<sup>8,17)</sup> Studies of Kaiser et al. on non-small cell lung cancer lines showed that 7/16 cell lines were positive for ERs content, 12/13 cell lines express specific RNA, and 5/17 cell lines were positive for estrogen receptor in immunohistochemistry. But from the other side, only 4 specimens from 52 tumors ERs were positive by immunohistochemistry. Using immunohistochemistry, progesterone receptor was detected in 13 of the 17 NSCLC cell lines, PCR method for RNA detection in 11 of the 14, and ligand binding method in 13 of the 16 NSCLC cell lines. Positive progesterone receptor expression was found in 23 of the 52 tu-

**Table 1. Estrogen and progesterone receptors expression in primary lung cancer patients determined by immunohistochemistry**

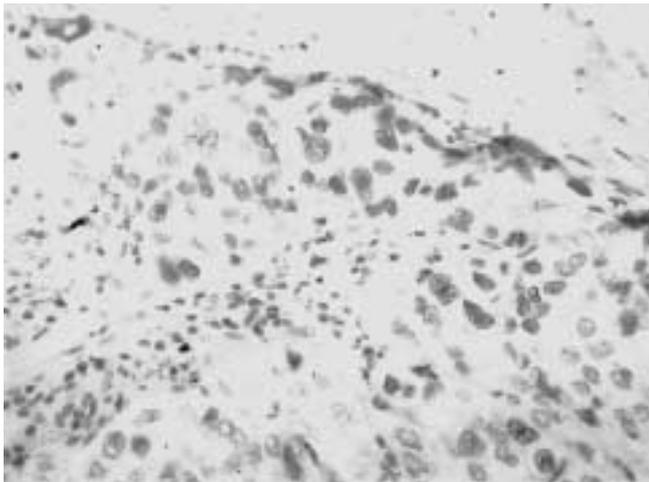
Number	Gender	Age	Histology	ER-R clone 1D5	ER-R clone 6F11	PR-R clone 1A6
1	M	61	adenocarcinoma	-	-	-
2	M	69	adenocarcinoma	-	-	-
3	K	61	adenocarcinoma	-	-	-
4	M	72	adenocarcinoma	-	-	-
5	K	59	adenocarcinoma	-	+	-
6	M	58	adenocarcinoma	-	-	-
7	M	78	adenocarcinoma	-	-	-
8	M	68	adenocarcinoma	-	-	-
9	M	57	adenocarcinoma	-	-	-
10	K	48	adenocarcinoma	-	-	-
11	M	58	squamous	-	-	-
12	M	52	squamous	+	-	-
13	M	65	squamous	-	-	-
14	M	62	squamous	-	-	-
15	M	66	squamous	-	-	-
16	M	61	squamous	-	-	-
17	M	65	squamous	-	-	-
18	M	70	squamous	-	-	-
19	M	68	squamous	-	-	-
20	M	64	squamous	-	-	-
21	K	42	adenocarcinoma	-	-	-
22	K	62	squamous	-	-	-
23	K	72	adenocarcinoma	-	-	-
24	K	56	adenocarcinoma	-	-	-
25	K	66	adenocarcinoma	-	-	-
26	K	56	adenocarcinoma	-	-	-
27	K	46	adenocarcinoma	-	-	-
28	K	69	adenocarcinoma	-	-	-
29	K	56	adenocarcinoma	-	-	-
30	K	63	squamous	-	-	-
31	K	73	squamous	-	-	-
32	K	60	squamous	-	-	-

mor samples.<sup>18)</sup> Cagle et al. by using the pre-labeled sucrose gradient method revealed very weak positive expression for estrogen receptors in 3/19 lung cancer tumors, and in 5/19 for progesterone. Receptor expression was found only in squamous cell carcinomas.<sup>17)</sup>

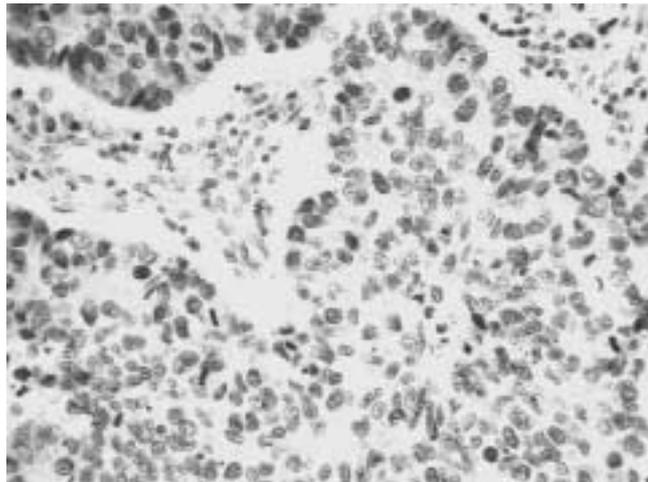
The good correlation between expression of estrogen related protein p29 and estrogen receptor expression was shown. Vargas et al. revealed positive immunoreactivity for estrogen related protein, p29 in 98% (109/111) of lung cancer specimens. Higher immunoreactivity for p29 was connected in this study with better prognosis for women but not for men with lung cancer.<sup>21)</sup>

Immunohistochemical staining with specific monoclonal antibodies is a commonly accepted method for detection of ER and PR receptors in the neoplastic tissue of breast, prostate and endometrium.<sup>9,25)</sup> This method is more specific than biochemical assay, because of the use of monoclonal antibodies reacting with epitopes which pos-

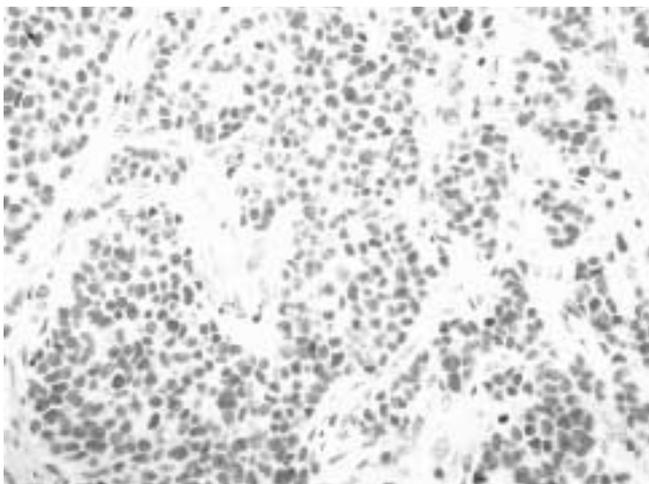
sess only minimal variability. Estrogen receptors were detected immunohistochemically in 96, 8% of lung cancer tumors by Canver et al. in 7, 1% by Ollayos and 0% by Vargas et al.<sup>8,18,21)</sup> Recently Di Nunno et al. showed no nuclear or cytoplasmic expression of ERs and PRs in the 248 non-small cell lung cancer specimens.<sup>20)</sup> Variety of technique and variety of clones of antibodies which were used are the possible cause of differences between authors. Thus to clarify discrepancies, we studied in non-small cell lung cancer tissues by two conventionally used monoclonal antibodies the expression of estrogen receptors and progesterone receptors by one monoclonal antibody. These standardized techniques with the use of monoclonal antibody directed against specific epitopes with heat-induced citrate antigen retrieval allows the detection of significant receptor expression in examined tissues. In our study only a very weak expression of ER receptors in one of 32 cases for each type of antibody in



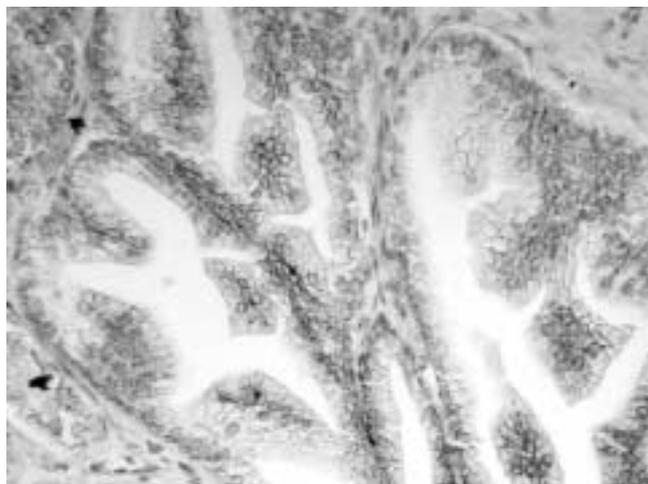
**Fig. 1.** Squamous carcinoma – weak nuclear staining by antiestrogen receptor antibodies ID5 (DAKO A/S, Glostrup, Denmark). (original magnification  $\times 200$ )



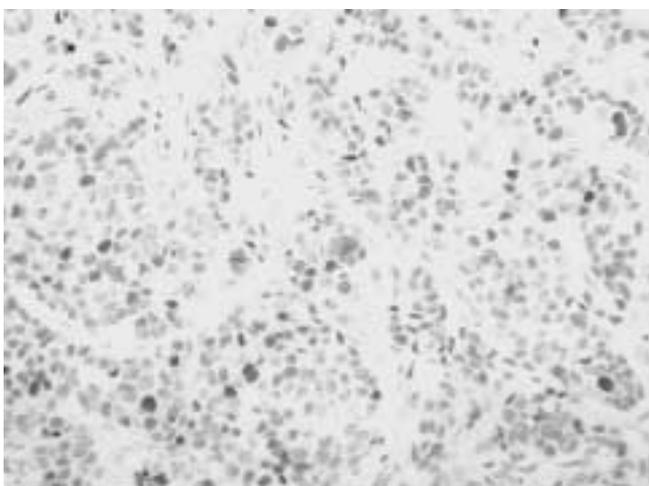
**Fig. 2.** Squamous carcinoma – negative estrogen receptors stain (ER-ID5). (original magnification  $\times 200$ )



**Fig. 3.** Normal breast tissue – estrogen receptors positive control (ER-ID5). (original magnification  $\times 200$ )



**Fig. 4.** Normal prostate tissue – estrogen receptors negative control (ER-ID5). (original magnification  $\times 200$ )



**Fig. 5.** Normal breast tissue – progesteron receptors positive control (PR-1A6). (original magnification  $\times 200$ )

different patients was found. None of the specimens from our patients expressed PR receptors. The results of our study support value of this method for histological differentiation of cancer specimens.

The role of routinely used monoclonal antibodies for detection of sex steroid receptors in lung cancer tissues seems to be established. It is very important for differentiation of cancer cells. The positive ER/PR receptors expression is an important argument against the pulmonary origin of unknown primary tumour.<sup>26)</sup>

But from the other side, conflicting results are quoted in the literature and there are some suggestions about the role of ER in lung carcinogenesis, so more studies for assessment of different genetic variants of estrogen receptor in lung tumor cells are needed.

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