Expression of Estrogen and Progesterone Receptors in Non-small-cell Lung Cancer Tissue Using Tissue Microarray Method

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조직 미세배열법을 이용한 비소세포 폐암 조직에서 에스트로겐과 프로게스테론 수용체 발현

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연구 배경 : 비소세포 폐암의 암화 과정에서 에스트로겐과 프로제스테론 단백의 역할에 대한 면역조직화학 염색을 이용한 연구들이 진행 중이다. 그러나 이 연구들은 아직 일치된 결과를 보이고 있지 않으며 이는 상용하는 면역조직화학 염색법이 한 문제로 제시되고 있다. 저자 들은 최근 새로 개발된 조직미세배열법을 이용하여 비소세포 폐암 환자의 조직에서 이들 호르몬 수용체 발현을 연구하였다.

대상 및 방법 : 대상은 70예의 비소세포 폐암 환자로 남성이 74%, 여성이 26%이었다. 이들의 포르말린 고정, 파라핀 포매 조직을 이용하여 조직미세배열을 구축하였다. 가열을 통한 항체 재생 후에 폐암 조직에서 일차 단일클론 항체 (ER1D5와 PR1A6)를 이용한 면역조직화학 염색을 시행하였다.

결 과 : 흡연력은 현재 흡연자가 49%이었고, 비흡연자와 금연자는 각각 27%와 24%이었다. 폐암의 조직학적 분류는 편평상피세포암이 34예이었고, 선암, 편평상피선암, 기타 세포형은 각각 24예, 9예와 3예이었다. 단일클론 항체를 이용한 염색에서 양성 결과를 보이는 비소세포 폐암 세포는 관찰되지 않았다.

결 론 : 미세조직배열법을 이용한 에스트로겐과 프로제스테론 수용체 연구는 모든 비소세포 폐암 조직에서 음성 결과를 보였다. 현재 면역조직화학 염색에 사용되는 호르몬 수용체가 비소세포 폐암 조직에서 발현이 되지 않을 가능성을 시사해주는 소견으로 향후 적절한 항체들을 이용한 추가적인 연구가 필요하겠다. (*Tuberc Respir Dis 2005; 58:54-58*)

Key words : Lung Cancer, Receptor, Estrogen, Progesterone

Introduction

In South Korea, a nation with one of the highest smoking rates in the world, lung cancer has become one of the leading cause of death among the population. Epidemiological studies showing that female smokers are at a higher risk of acquiring lung cancer than male smokers suggest that a person's gender may be a determinant in their susceptibility to cancer[1,2,3]. To answer this question, steroid hormones have been studied. Receptors of steroid hormones have been known to play a critical role in normal lung growth and development[4,5]. Furthermore, the role of steroid hormones in the development of lung cancer has been suggested in epidemiologic and experimental studies[6,7]. Expression of estrogen receptors (ERs) and/or progesterone receptors (PRs) has been studied in paraffin-embedded, non-small-cell lung cancer (NSCLC) tissue using conventional immunohistochemical (IHC) studies which showed the inconsistent rates for positive results[8-15]. Recently, tissue microarray (TMA) has been developed and used extensively for analysing molecular markers at the gene or protein level, with benefits in terms of the time and cost[16,17]. In this study, we constructed TMA from formalin-fixed,

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paraffin-embedded tissues of NSCLC patients and investigated expressions of the ER and PR with monoclonal antibodies commonly used.

Matenals and Methods

We performed the study with paraffin-embedded tissues from 70 patients who were diagnosed as NSCLC in a tertiary hospital and received the surgical resections with radical mediastinal lymph node dissection from February 1997 to March 2002. Construction of TMAs was achieved by acquiring 2mm cylindrical core specimens (non-tumor, tumor, avail-able metastatic tumor in lymph node) from paraffin-embedded tissue of 70 primary lung cancers and 43 metastatic mediastinal lymph nodes. The general procedure consisted of two sections, 4μm thick, from each block of TMAs. Sections were mounted on glass slides, which were prepared for staining in a standard fashion by oven heating, deparaffinizing and microwave boiling in citrate buffer (antigen retrieval). The slides were stained by using the antibodies clone 1D5 (monoclonal, 1:50, Dako, Glostrup, Denmark) for ER and clone 1A6 (monoclonal, 1:50, Dako, Glostrup, Denmark) for PR and a standard avidin-biotin complex method with diaminobenzidine. The positive and negative controls for both antibodies were paraffin blocks that contained benign and malignant breast tissue, respectively. The cases showing nuclear staining for ER and PR were interpreted as positive.

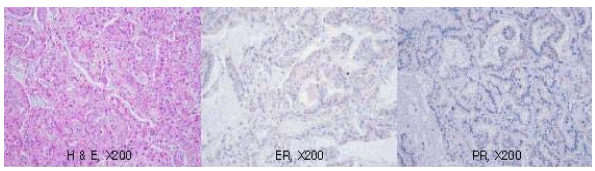

Figure 1. Immunohistochemical stain on an adenocarcinoma tissue showed negative results for estrogen receptor (ER) and progesterone receptor (PR).

Results

Histologic specimens from 52 (74%) men and 18 (26%) women with a mean age of 63 years old were used for this study. There were 34 (49%) current smokers, 19 (27%) non-smokers, and 17 (24%) former smokers. There was only one female smoker with

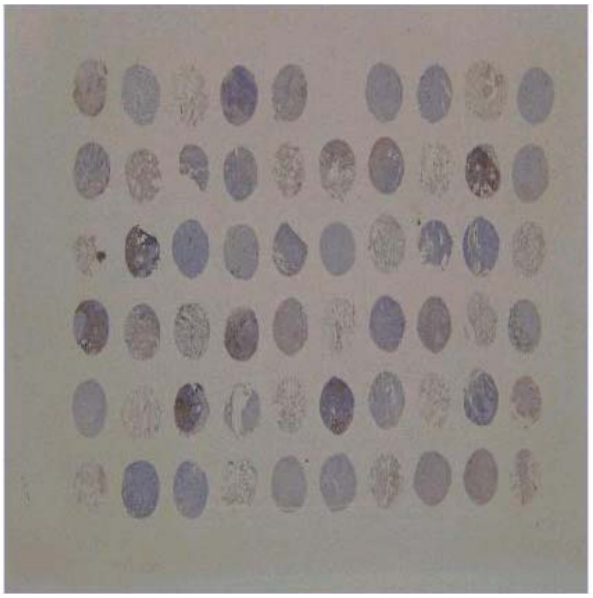

Figure 2. Negative estrogen receptor (1D5) stainings on tissue microarray

Figure 3. Negative progesterone receptor (1A6) stainings on tissue microarray

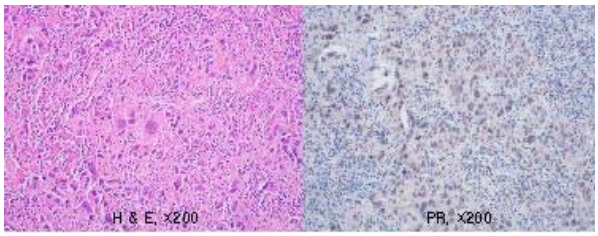

Figure 4. Immunohistochemical stain on mediastinal lymph nodes from a patient of adenocarcinoma showed positive result for progesterone receptor (PR).

two pack-years. The pathological stage of each patient according to the lung cancer staging system was as follows: IA in 9 patients, IB in 18 patients, IIB in 13 patients, IIIA in 19 patients; IIIB in 6 patients; IV in 5 patients[18]. Thirty-four patients had squamous cell carcinoma, 24 patients had adenocarcinoma, 9 had adenosquamous cell carcinoma, and one of each had bronchoalveolar carcinoma, carcinosarcoma and pleomorphic carcinoma. The results of IHC stainings for both ER and PR were negative in all paraffin-embedded tissues of NSCLC (Figs. 1, 2 and 3). Results of IHC staining for ER were negative in all mediastinal lymph nodes. IHC staining for PR was positive in mediastinal lymph node metastasized in two male patients, one with a squamous cell carcinoma and one with an adenocarcinoma (Fig. 4).

Discussion

A gender difference has been suggested in the carcinogenesis and progression of lung cancer. Steroid hormones have recently been implicated in playing a role in this difference. Advances in the field of molecular biology of cancer, estrogen and progesterone has known to be possible tumor growth factors with insulin-like growth factor-1, bonbesin/gastrin releasing peptide, transforming growth factor-α, retinoic acid receptor and opioid and nicotinic receptor[19]. Smoking can change the metabolism of estrogens into more carcinogenic forms and the use of exogenous estrogen has been shown to be related to lung cancer risk[7,20].

To date, conventional IHC studies for ERs and PRs in NSCLC tissue have been reported with various positive rates: 0-96.8% for ERs, and 0-34.7% for PRs (Table 1)[8-15,21]. Recently five IHC studies have

Table 1. Previous Reports of Estrogen Receptors (ER) and Progesterone Receptors (PR) Expression in Non-small-cell Lung Cancer Using Immunohistochemical Study with Formalin-fixed, Paraffin-embedded Tissue

Author, year	Antigen	Positive Rate(%)	Clone	HIER*
Ollayos, 1994	ER	7.1	not stated	pronase
	PR	-	-	-
Canver, 1994	ER	96.8	not stated	not stated
	PR	21.8	not stated	not stated
Su, 1996	ER	6.1	not stated	yes
	PR	34.7	not stated	yes
Vargas SO, 1998	ER	0	1D5	yes
	PR	-	-	-
Omoto Y, 2001	ER	0	1D5	yes
	PR	-	-	-
Di Nunno, 2000	ER	0	1D5	yes
	PR	0	hPRa3	yes
Dabbs, 2002	ER	0	1D5	yes
		66.6	6F11	yes
	PR	-	-	-
Radzikowska, 2002	ER	3.1	1D5	yes
		3.1	6F11	yes
	PR	0	1A6	yes
Present Study, 2004	ER	0	1D5	yes
	PR	0	1A6	yes

*heat-induced epitope retrieval

shown negative results for ERs or PRs[11-15]. However it is early to conclude that ERs or PRs do not play a role in the carcinogenesis of lung cancer based on these results, because there are discrepancies in the results of IHC and western blot studies of NSCLC tissue. These could be explained by the possible decay of antigenicity during tissue processing, the alteration of target antigenic structure by formalin fixation and the presence of ER of not full length but variant forms in lung cancer tissue which is different in breast cancer[11,12,22,23]. Therefore, current IHC study with ER and PR may be clinically useful in differential diagnosis when the lung cancer origin is confused1[5]. Genetic variants of ERs or PRs in lung cancer still remain to be clarified.

The interpretation of the inconsistent IHC results from these studies is not an easy task. Inconsistencies resulted from which fixation method was used, whether the clones of the monoclonal antibodies were identified or not and different ratios of the dilution and antigen retrieval. The conventional IHC staining method has a shortcoming in the absence of standardized positive and negative values with the disagreement of interpretation among pathologists[8]. The method of TMA was developed by Kononen *et al*. in 1998[16]. Because TMA consists of a slide containing hundreds or thousands of cases, it makes the analysis of molecular markers in a large number of cases easier, cheaper and faster than those in the conventional IHC method. Therefore, TMA has an advantage for irregular staining problems, where different conditions are applied to each slide in the conventional IHC staining method[16,24]. However TMA has not yet been applied to study the expression of ER and PR in cancer tissue. In this respect, TMA could be a useful tool in IHC study for the expressions of ER and PR. Our results using TMA were similar to those of recent studies performed with advanced IHC techniques such as the effective antigen retrieval method which showed negative results for ER1D5 and PR1A6[11-14].

Summary

Background :

To evaluate the role of estrogen and progesterone in the carcinogenesis of NSCLC, IHC studies for the expression of the receptors of estrogen and progesterone have been performed with inconsistent results. Recently the TMA method has been developed and has become recognized as a useful and rapid method for extensively analysing molecular markers at the gene and protein level. We have investigated their expressions in the tissue from NSCLC using the microarray method.

Methods :

The TMA construction was made with 70 formalin-fixed, paraffin-embedded tissues of NSCLC. After heat-induced epitope retrieval, IHC staining on primary tissues of NSCLC was performed with the monoclonal antibodies, ER1D5 and PR1A6.

Results :

Our sample of 70 consisted of 74% men and 26% women. Of the patients, 49% were current smokers, 27% were non-smokers and 24% were former smokers. By histologic classification, 34 patients had squamous cell carcinoma, 24 had adenocarcinoma, 9 had adenosquamous cell carcinoma, and 3 had other carcinomas. No cancer cells were immunostained with these monoclonal antibodies in any primary tissues of NSCLC.

Conclusions :

No expression of neither of the two receptors was found in any of the lung cancer tissues. This suggests that adequate genetic variants for IHC staining need to be developed for NSCLC.

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