□ 원 저 □

Clinical Study of Neuron Specific Enolase (NSE) in Small Cell Carcinoma of the Lung

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=국문초록=

미분화소세포 폐암에서의 Serum Neuron Specific Enolase (SNSE)의 변동에 관한 연구

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암표식자 임상연구의 일환으로 신경세포 및 APUD계 세포에서 특징적으로 증가한다고 알려진 NS의 혈청농도를 51명의 소세포암환자군과 31명의 대조군에서 방사면역측정법으로 측정하여 다음과 같은 결과를 얻었다.

1) 정상대조군 15명, 비폐암군 6명, 비소세포암군 10명에서 측정한 SNSE 평균농도는 각각 9.2 ± 5.4 ng/ml, 7.3 ± 3.2 ng/ml, 13.9 ± 5.8 ng/ml 였다.

2) 소세포암환자군에서의 SNSE 평균농도는 43.0 ± 30.4 ng/ml로서 대조군에 비하여 유의하게 증가되었다.

3) 소세포암 환자군에서의 Limited Disease의 평균농도는 27.4 ± 15.2 ng/ml, Extended Disease에서는 49.8 ± 32.8 ng/ml로 소세포암의 진행정도에 따라 SNSE의 농도가 증가함을 보여주었다.

4) 화학요법치료후 부분관해 또는 완전관해가 온 환자의 SNSE 평균농도는 8.0 ± 6.1 ng/ml로 정상치와 통계학적으로 유의한 차이가 없었으며 치료에도 불구하고 암이 진행된 환자의 SNSE 평균농도 97.6 ± 39.7 ng/ml는 치료전의 환자군에 비하여 증가되어 있었다.

5) 이상의 결과로 SNSE 측정은 소세포암의 진단 및 진행정도의 판정뿐만 아니라 치료에 대한 반응을 판정하는 암표식자로 유용하게 사용될 수 있음을 알 수 있었다.

Introduction

Histologic classification of lung cancer is becoming more important in clinical practice due to wide therapeutic application of cancer chemotherapy and radiation therapy. The classification

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which was adopted by the W.H.O. Committee(1980) include (1) Squamous-cell carcinoma (Epidermoid carcinoma), (2) Small-cell carcinoma, (3) Adenocarcinoma, (4) Large cell carcinoma, (5) Adenosquamous carcinoma, and this classification was designed by the observed tumor cell appearances as seen by light microscopy and known to make no attempt to base the histogenetic classification.

Among the above variety of histologic types of lung cancer, small-cell carcinoma comprises about 20-25% of total lung cancer and has been known

Table 1. Patient Categories & its Distribution

Patient categories	Subclassification
SCLC (51)	Limited disease (23) Entended disease (28)
NSCLC (10)	Squamous cell ca (7) Adenocarcinoma (3)
Other cancer (6)	Gastrointestinal tract tumor (3) Lymphoma (2) Meningioma (1)
Normal subjects (15)	
Total	82

() :Number of cases

SCLC :Small cell lung cancer

NSCLS : Non-small cell lung cancer

as the most rapidly progressing variety of lung cancer and also being the one of the most sensitive cancer to anti-cancer chemotherapy^{1~2}). Another unique aspect of this small-cell lung carcinoma is its histogeneic relation with APUD (Amine Precursor Uptake and Decarboxylation) and its capability to produce paraneoplastic syndrome by APUD enzymes, hormones and polypeptides substances^{3~4}).

These kinds of biologic substances which derived from tumor has been studied extensively as a possible tumor marker⁵) and many attempts have been made to correlate with clinical stage, diagnosis, therapeutic response and for the detection of recurrence of various carcinomatous diseases⁶).

Neuron Specific Enolase is one another kind of neuron specific protein and has been known to be increased among the patients with non-functioning islet cell tumor⁷), small-cell carcinoma⁸) and neuroblastoma⁹).

The following study attempted to evaluate the diagnostic and prognostic significance of serum

Neuron specific Enolase(NSE) for the small-cell carcinoma of the lung among the Korean population.

Materials and methods

The group under study includes 51 patients with small-cell lung cancer and 31 control subjects including 15 normal, 6 other types of cancer and 10 non-small cell cancer of the lung(Table 1). All patients under study were histologically proven cancer patients by bronchofiberscopic biopsy, percutaneous needle aspiration biopsy, pleural biopsy, lymph node biopsy or cytologic examination of sputum and pleural fluid¹⁰).

Clinical staging of small-cell lung carcinoma was made into simple two stage system of Limited disease(LD) and Extended disease(ED)¹¹). LD category was 23 subjects and ED category was 28 patients.

Treatment modality comprises chemotherapy of VP-16, Ifosfamide and Cisplatin/Cyclophosphamide, Adriamycin and Vincristin alternative treatment and radiation therapy for LD and chemotherapy only for ED.

The criteria of treatment response was determined into 4 catgories as complete remission(CR), partial remission(PR), stationary disease(SD) and progressive disease(PD)(Table 2).

The assay of serum Neuron Specific Enolase was made by EIKEN radioimmunoassay kit, supplied by EIKEN Immunochemical Laboratory. The principle of assay is based on the immunologic reaction using I ¹²⁵-labeled NSE tracer and NSE standard curve. All serum samples were kept frozen at -20°C until assay procedure undertaken and

Table 2. Criteria of Response

Complete remission (CR)	Disappearance of all clinical evidence of disease
Partial remission (PR)	Reduction by at least 50% in the sum of all measurable disease
Stable disease (SD)	Reduction by less than 50% in the sum of all measurable disease or no change
Progressive disease (PD)	Any increase in size of measurable disease, or appearance of new disease

Table 3. Comparison of SNSE Titers in Various Categories of Patients

Patient categories	Mean $\pm$ SD(ng/ml)
SCLC	43.0 $\pm$ 30.4
Limited disease	27.4 $\pm$ 15.2
Extended disease	49.8 $\pm$ 32.8****
NSCLC	13.9 $\pm$ 6.7***
Other cancer	7.3 $\pm$ 3.2**
Normal subjects	9.2 $\pm$ 5.4*

SCLC:Small cell lung cancer

NSCLC:Non-small cell lung cancer

* Comparison between SCLC and Normal Subjects($P<0.005$)

** Comparison between SCLC and other Cancer ($P<0.01$)

*** Comparison between SCLC and NSCLC($p<0.005$)

**** Comparison between subgroups of SCLC ($p<0.05$)

Fig. 1. SNSE titers in various categories of patients. (SCLC:Small cell lung cancer NSCLC:Non-small cell lung cancer)

were assayed duplicates.

The statistical analysis was done using Student's t-test.

Results

1) Serum Neuron Specific Enolase(SNSE) con-

Fig. 2. SNSE titers in subgroups of small cell Lung cancer.(LD: Limited disease, ED: Extended disease)

were assayed centration of the normal control subjects($N=15$)was 3.0~19.0 ng/ml(9.2 ± 5.4 ng/ml). Among the study group, they showed 43.0 ± 30.4 ng/ml in small-cell carcinoma of the lung($N=51$), 13.9 ± 6.7 ng/ml in non-small cell carcinoma of the lung($N=10$) and 7.3 ± 3.2 ng/ml in other nonpulmonary cancer($N=6$), respectively(Table 3, Fig. 1). The small-cell lung carcinoma group showed significant higher value compared with other group of normal control, non-small cell lung cancer and other cancer group.

2) In comparison between subgroups of small-cell lung carcinoma, limited disease showed 27.4 ± 15.2 ng/ml and 49.8 ± 32.8 ng/ml in extended disease which is also significantly higher in extended disease group(Table 3, Fig. 2).

3) SNSE was also measured at pre and post-chemotherapy of small-cell lung carcinoma for the comparison between the disease progression. Eighteen subjects who have clinical remission(partial or complete) showed 8.0 ± 6.1 ng/ml and 5 subjects who have disease progression in spite of chemotherapy showed 97.6 ± 39.7 ng/ml which is significantly higher than the pre-treatment values (Table 4, Fig. 3,4).

Table 4. Comparison of SNSE Titers Before and After Treatment of Small Cell Lung Cancer

Patient categories	Mean±SD(ng/ml)
Before treatment	43.0±30.4
After treatment	
Remission	8.0± 6.1*
Progression	97.6±39.7*

* Comparison between pretreatment and posttreatment group(p<0.005)

Fig. 3. SNSE titers in various states of small cell lung cancer.

Discussion

It has been known that malignant cells produce hormones, proteins, enzymes and many kinds of cellular metabolic products. Some of them are used as tumor marker for early detection, diagnosis and staging of malignancy and evaluation of treatment¹²⁾. Also there have been trials to use them tagged to anticancer agents as treatment vehicles.

But, there are requirements for these materials to be used as tumor marker. First, they should be able to differentiate malignancy from normal or benign conditions. Second, they should have

Fig. 4. Comparison of SNSE titers at the time of diagnosis and during remission.

high sensitivity and specificity. Third, they should have organ specificity. Also, to be used clinically, they should be used for differential diagnosis from other diseases and for follow-up of treatment.

But, unfortunately, no material has fulfilled these requirements and some materials which fulfilled some of these requirements have been used clinically. Since Goldberger and Freedman found CEA(Carcinoembryonic antigen) in embryonal cells and colon cancer in 1965, CEA has been used as a convenient and sensitive method to predict the recurrence of cancer after colon cancer surgery¹³⁾. α -feto protein in primary liver cancer, HCG(Human Chorionic Gonadotropin) in trophoblastic tumor and germ cell tumor, and acid phosphatase isoenzyme in prostate cancer are examples used in clinical medicine.

In the case of lung cancer, many tumor markers were tried, but could not be used widely because of their low specificity¹⁴⁾. On the other hand, since NSE was found to increase in neuroendocrine and APUD tumors¹⁵⁾ there have been many researches on the utility of NSE in small-cell lung cancer which is known to be originated from APUD system^{8,16)}.

Neuron Specific Enolase is acid protein which Moore et al separated in bovine brain cells using DEAE cellulose chromatography and electrophoresis in 1965¹⁷⁾. Bennet et al found α -antigen which was present in rat brain cells specifically and reported that it was different from material found by Moore¹⁸⁾. But Marangos et al confirmed that these two materials were identical¹⁹⁾. Marangos also found that these material was present only in neuron but not in glial cells. So this material was called Neuron Specific Protein^{20,21)}. Soon it was found to have enolase activity.

In 1980 Hullin et al found NSE in human brain by radioimmunoassay and subsequently in almost every tissue of human body²²⁾. But concentration of NSE in other tissues were less than 3% of the brain and was thought to be the result of nerve supply. But, because NSE was found in RBC's which have no innervation, the explanation was not verified²³⁾.

In 1978 Schmachel et al found NSE in APUD cells and subsequently Prinz et al noticed increase of NSE in non-functioning islet cell tumor²⁴⁻²⁶⁾. Because small-cell lung cancer is from APUD system, application of NSE in this disease was tried by Carney et al. He reported that NSE could be used as a tumor marker in small-cell lung cancer and similar results were reported by Akoun²⁷⁾ and Essher²⁸⁾.

The authors measured serum NSE concentration in normal subjects, small-cell lung cancer patients, non-small cell lung cancer patients and other cancer patients. Mean serum concentration in small cell lung cancer patients was significantly raised compared with other groups. And ED of small-cell lung cancer patients group the mean concentration of NSE was significantly higher than that of LD. This means that serum NSE increases according to the degree of tumor progress and the results were similar to those of Carney, Ariyoshi, Akoun and Essher²⁹⁾.

We tentatively took upper limit of normal value as 20 ng/ml which is mean plus 2X standard deviation. According to this value the sensitivity

was 72.7% and the specificity was 93.6%. In LD 6 patients out of 10(60%) had serum NSE value above 20 ng/ml and in ED 18 patients out of 23 (78.3%) had value above 20 ng/ml. In normal subjects and other cancer group there was none who had NSE concentration above 20 ng/ml and in non-small cell lung cancer group only 2(20%) had value above 20 ng/ml. And in small cell lung cancer patients, the patients whose value were above 60 ng/ml were all ED patients. According to this result we could find that NSE could be used for diagnosis and staging of small cell lung cancer.

The mean serum NSE concentration of the patients who had partial or complete remission after chemotherapy or radiation therapy decreased to the value similar to the normal subjects. On the other hand, the mean serum NSE concentration of the patients whose disease progressed despite of treatment was remarkably raised. So we concluded that NSE is a useful tumor marker for monitoring the clinical course during treatment of small-cell lung cancer.

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