Expression of the somatostatin receptor family mRNAs in lung cancer

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Abstract To investigate the characteristics of expression and distribution of 5 subtypes of somatostatin receptors (SSTR1~5) in lung cancer, *in situ* hybridization was used to examine the expression patterns of SSTR mRNAs in 21 cases of different pathologic types of lung cancer tissues with [α-^{35}S]dATP labeled oligonucleotides of the 5 SSTR subtypes as probes. Additionally, Leica Q-500 image analysis processing system was employed for the semi-quantitatively analysis of the hybridization signals. Patterns of SSTR1~5 expression in lung cancer tissues were found as follows. SSTR2 was prominent in small cell lung cancer (SCLC), whereas in non-small cell lung cancer (NSCLC) including the adenous cancer (Ad) and the squamous cancer (Sq), the expression of SSTR1 mRNA was stronger than that of the other 4 types. The expression density of SSTR1~5 in the NSCLC was higher than the SCLC ($p < 0.01$). The expression patterns and densities of the SSTR subtypes showed heterogeneity in different pathologic types of lung cancer. The expressions of the SSTR mRNAs in both SCLC and NSCLC indicated the positive prospects for somatostatin analog(SSA)-oriented agents in the treatment of both types of the lung cancer.

Keywords Somatostatin receptor, Lung neoplasms, *In situ* hybridization

CLC numbers Q786, R817.1, R734.2

1 INTRODUCTION

It is known that somatostatin receptors express in different types of tumors with high density. There are many reports about the expressions of SSTR1-5 mRNAs in various types of pituitary tumors, but little was known about their expressions in lung cancer. Accordant conclusions have been obtained from many studies about the presence of SSTR in SCLC. However, it is controversial whether SSTR expressed in the NSCLC[1,2]. In the present study, radioactive *in situ* hybridization was applied to examine the characteristics of the distribution and expression of SSTR mRNAs in the lung cancer. The purpose of present study is to provide evidences for the selection of somatostatin analog-oriented agents which can specifically bind to certain subtype of SSTR and inhibit proliferation of lung cancer.

Supported by the National Natural Science Foundation (39500035)
Manuscript received date:2000-07-17

2 MATERIALS AND METHODS

2.1 Tissue preparation

Twenty one cases of tumor tissues were collected from Xijing Hospital and Tangdu Hospital, The Fourth Military Medical University. Among them, 15 cases were NSCLC (including 8 cases of Ad and 7 cases of Sq) and 6 cases were SCLC. All the specimens were verified by histopathological tests. The tissues were washed with saline, briefly frozen in liquid nitrogen and stored at –80°C.

2.2 Preparation of oligonucleotides probes

Oligonucleotide probes used in the present study were synthesized with DNA synthesizer (Cybersyn BJ Co.). All of the SSTR1~5 probes consisted 45 bases complementary to the bases coding for amino acides 252~267 237~252, 366~381, 47~62, 274~289 of the human SSTR1~5 mRNA, respectively[3,5]. The probes were labeled with [α-^{35}S]dATP (>37 TBq/mmol, Amersham) by a 3'-end-labeling method and purified with nucleotide depurative column, giving the specific activities of 6.5~10 TBq·g^{-1}.

2.3 *In situ* hybridization

Frozen tumor tissues were cut into sections at 10~15 μm on a cryostat (Bright UK) and thow-mounted on gelatin-coated slides. The secyions were then fixed in 4% paraformaldehyde for 20~30 min, rinsed in 0.1 mol/L phosphate-buffered saline, treated with prehybridization treatment solution, dehydrated in alcohol and delipidated in chloroform. 200 μL of hybridization solution containing 15μL of dithiothreitol and 10~15 MBq labeled probes were carefully added onto the surface of each tissue section. The sections were then incubated under moist condition for 24 h at 37°C. After washed with 1-standard citrate saline buffer (SSC), sections were dipped in Konica NR-M2 nuclear-emulsion. Sections were then developed with Kodak D19 developer and fixed with F-5 fixer after exposed for 4~6 weeks in the dark box at 4°C. The sections were counterstained with thionin, coverslipped with neutral gum and observed under light microscope.

2.4 Competitive control test

Parts of sections from various lung cancer tissues were randomly selected and pre-incubated with hybridization solution containing excess amount of unlabeled probes. The pre-incubated sections were then rinsed and incubated again with hybridization solution containing normal amount of labeled probes. No hybridization signals were observed, indicating the specificity of the probes used in the present study.

2.5 Semi-quantitative analysis

From each subtype of lung cancer tissues, 4 sections were randomly selected for the transparency analysis of the hybridization signals by Leica-Q500 Image Analysis Processing System (Germany). The transparency value of the background area of each

section was regarded as 100, against which the relative transparency of each section was obtained.

2.6 ANOVA method was used in the statistic analysis

3 RESULTS

The positive rate of SSTR1~5 in lung cancer tissues are shown in Table 1. The distribution patterns of 5 subtypes of SSTR varied in different tissue types of lung cancer and also showed individual differences greatly. SSTR1 was dominantly present in NSCLC (including Ad and Sq) while SSTR2 mainly distributed in SCLC.

Table 1 Positive rate of SSTR1 ~5 mRNA distributions in various lung cancer tissues (%)

Lung cancer type	Number of cases	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Ad	8	75(6)[1]	63(5)	63(5)	75(6)	63(5)
Sq	7	86(6)	43(3)	43(3)	43(3)	71(5)
SCLC	6	33(2)	83(5)	17(1)	33(2)	50(3)

[1]Data in the () indicate the number of positive cases

Table 2 shows the semi-quantitative analysis of the expression densities of SSTR1~5 mRNAs. The lower relative transparency values represented for the stronger hybridization signals. The expression density of each subtype mRNA of SSTR in NSCLC was higher than that in SCLC. ANOVA results showed that the expression densities of SSTR1, SSTR4 and SSTR5 in Ad and Sq were significantly different ($p < 0.01$) from that in SCLC. No significant difference ($p > 0.05$) existed between the densities of SSTR2 and SSTR3 in Ad and that in Sq.

Table 2 Relative transparency of SSTR1~5 in various types of lung cancer tissues (%)

Lung cancer type	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Ad	85.2±1.4[1]	82.2±2.2	79.7±1.5	80.4±1.8	85.9± 1.9
Sq	82.1±1.7	82.2±1.8	79.9±1.4	77.8±1.9	78.9± 1.2
SCLC	93.7±1.8	90.6± 1.1	98.3± 1.7	99.4± 1.7	93.8±1.4

[1]Data are expressed as mean $\pm SE$

4 DISCUSSION

Somatostatin and its analogs exert multiple physiological and pharmacological functions through SSTR subtypes. Their physiological and pharmacological functions include neurotransmission or neuroregulation in the central nervous system, hormone-like regulation in the peripheral tissues, directly or indirectly inhibition of tumor cells' proliferation and differentiation, etc. Before 1992, when SSTR subtypes were firstly identified, it was the distribution of overall SSTR but not the detailed distribution of each subtype that were observed by using the methods of autoradiography and receptor scintigraphy with iodinated somatostatin analogs (SSA) in lung cancer[6]. Using autoradiography method,

Sagman *et al*[1]. reported that 2 of 3 cases of SCLC were SSTR-positive, while all the 5 cases of NSCLC were SSTR-negative. According to this result, it was believed that there was no expression and distribution of SSTR in NSCLC. However, O'Bynre *et al*[2]. in 1994 successfully detected 3 cases of SSTR-positive NSCLC by using ^{111}I-pentetreotide receptor scintigraphy. Using *in situ* hybridization technique, which is more sensitive than autoradiography and scintigraphy, we successfully detected the expression of SSTR1~5 mRNA in the present study. Comparison of SSTR's distribution and expression density in SCLC and NSCLC indicated that SSTR1 was dominantly present in NSCLC while SSTR2 in SCLC. Semi-quantitative analysis of expression density showed that the content of SSTR in NSCLC was much higher than that in SCLC (p <0.01). These findings support the blueprint that SSA can be used in the targeted treatment to not only SCLC but also NSCLC[2].

Also using *in situ* hybridization method, Reubi *et al*[7] found only SSTR2 expressed in 2 cases of SCLC. Our study suggested that there still exist SSTR subtypes besides SSTR2 in SCLC. Reubi *et al*[8]. conferred the mechanism of high expression of various subtypes in lung cancer tissues based on his further studies. It may be that SSTR, which is very little and almost undetectable with available techniques in normal human lungs, is intrigued to "re-express" in some neuroendocrine cells by their malignancy, which finally results in a high level of SSTR. The clarification of the distribution and expression level of various subtypes of SSTR in lung cancer tissues makes it possible to select high specificity and strong affinity SSA, labeled with radionuclide, as ligand, and to use it in receptor scintigraphy and receptor-induced target treatment of lung cancer.

5 CONCLUSIONS

The expression patterns and densities of SSTR subtypes showed different characteristics in various pathologic types of lung cancer. The expressions of SSTR mRNAs in both SCLC and NSCLC indicated the positive prospects for somatostatin analog-oriented agents in the treatment of both types of the lung cancer.

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