Expression and Clinical Significance of Somatostatin Receptor Subtype SSTR2,3 in Lymphoma

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ABSTRACT Objective: To investigate the expression of somatostatin receptor subtypes SSTR2 and SSTR3 in different types, different parts of lymphoma. Methods:The expressions of SSTR2 and SSTR3 gene in 105 cases lymphoma paraffin-embedded specimens were detected by RT-PCR. Results: The positive expression rate of SSTR2 and SSTR3 in the mucosa-associated lymphoma was (8/27), (6/27), diffuse large B-cell lymphoma (14/36), (12/36), NK / T cells lymphoma (9/22), (6/22), and Burkitt's lymphoma (6/20), (7/20). The total positive expression rate of SSTR2 were (37/105), and SSTR3 (31/105). In which the lesions in the diaphragm the total positive expression rate of SSTR2 and SSTR3 were (24/105), (19/105). The subdiaphragmatic total positive rate respectively (14/105),(11/105). Conclusion: We found that partial lymphoma at least express a somatostatin receptor of SSTR2,SSTR3, but the expression rates was low. However lymphomas are highly radiosensitive tumors whether low expression of somatostatin receptors in lymphoma is sufficient for diagnosis and targeting treatment in these tumors needs further study.

Key words: Somatostatin; Receptor; Lymphoma; RT-PCR

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Introduction

The somatostatin which is a cyclic peptide hormone synthesized by neuroendocrine cells or many other cells has a variety of biological functions. Related studies have shown that somatostatin and its analogues inhibit the growth of many tumors such as colorectal cancer, pancreatic cancer, liver cancer. The role of inhibition tumor growth is mediated by the somatostatin receptor (SSTR) in the tumor tissue. The effect of somatostatin receptor expression in tumor tissue on the treatment effect of somatostatin and its analogues was unkown. The SSTR have five different subtypes, including SSTR1-5. Some studies have shown that lymphoma express SSTR2, 3 only [1]. This study was to detect the SSTR2 and SSTR3 gene expression in 105 cases of lymphoma by RT-PCR, to provide a theoretical basis for lymphoma, diagnosis and treatment by somatostatin and its analogues.

1 Materials and methods

1.1 Clinical data

Collected 105 Paraffin-embedded specimens of patients which were diagnosed lymphoma in the Affiliated Hospital Of Medical College Qingdao Universitybetween January 1st, 2009 to July. 31,2010, and collected these clinical and pathological data, including gender, age, anatomical site, lesion distribution, and clinical stage. There are 46 cases of male, and female 59 cases, aged 35-73 years old, median age 59 years old.27 cases of mucosa-associated lymphoma, 36 cases of diffuse large B-cell lymphoma, 22cases of NK / T cell lymphoma, 20 cases of Burkitt's lymphoma, 54 cases of pathological parts at the diaphragm, diaphragmatic 51 cases.

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1.2 Methods

1.2.1 Extraction RNA from Lymphoma Paraffin specimens Concrete steps:(1)Section:a thickness of about 5-10 μm.(2)Xylene /ethanol dewaxing. (3)Digestion and purification. (4)preservation: Stored at -70 ℃.

1.2.2 PCR Purity and quantity of the RNA was assessed by spectrophotometry(A260/280 greater than 1.8). Then total RNA was reverse transcripted into cDNA by using the Prime Script RT-PCR kit (TaKaRa Bio Inc., Shiga, Japan), according to the manufacturer's instructions. The primer sequences for the human gene were shown in table 1. Primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai,China). The PCR reactions in duplicate were subjected to an initial denaturation at 94℃ for 2 minutes, followed by 40 cycles, denaturation at 95℃ for 30 seconds, annealing (SSTR2 57℃, 30seconds, SSTR3 63℃, 60seconds) and extension at 72℃ (SSTR2 40seconds, SSTR3 60seconds), extension at 72 ℃ for 5minutes (SSTR3 7minutes), cooling for 5 minutes at 4℃.

1.2.3 Control (1)Negative control: the composition of the reaction system and reaction conditions were the same as the experimental group, only without RNA. (2) Positive control: In this experiment as a positive control experiment using the RNA.(3) In order to determine the product of PCR amplification from cDNA (complementary DNA) template instead of the genome, each specimen by RT-PCR reaction without reverse transcriptase(the rest of the composition and reaction conditions and the experimental group the same).(4) Repeat the experiment: Test experimental reproducibility, the same specimens at least twice (at different times).

1.3 Results judgment

Agarose gel electrophoresis: the PCR reaction products in ag-

arose gel electrophoresis, to observe whether it is size of the target genes of 269bps and 264bps.

2 Result

The positive expression rate of SSTR2 in lymphoma tissues was 35.2%, and SSTR3 29.5%, at least one kind of receptor positive expression rate was 40%.

Table 1 Primers Used for RT-PCR

Primer	Sequence (5'-3')	Size (bps)
SSTR2 forward	CATCGTAGTGGCTGTCTTCATC	269
SSTR2 reverse	GGGATTTGTCCTGCTTACTGTC	
SSTR3 forward	GGCATCTTGATCTCTCTGGTG	
SSTR3reverse	GATGCTGGTGAACTGGTTGAT	264
β-actin f	TGACGTGGACATCCGCAAAG	205
r	CTGGAAGGTGGACAGCGAGG	

Table 2 SSTR2 and SSTR3 expression in four kinds of different types lymphoma

	SSTR2		SSTR3	
Types	(+)	(-)	(+)	(-)
MALT	8	19	6	21
DCBLC	14	22	12	24
NK/T	9	13	6	16
Burkitt	6	16	7	13

Table 3 The relationship between the SSTR2,3 expression and the lesion

Lesion	SSTR2		SSTR3	
	(+)	(-)	(+)	(-)
Diaphragm	24	30	19	35
Subphrenic	14	37	11	40

3 Discussion

SSTR belongs to the G protein-coupled receptor family, glycoprotein with seven transmembrane segments, by the seven transmembrane α2 helix, N2 end of the extracellular, C2 end of the Intracellular and extracellular loop and intracellular cyclic which connections of these structures [2]. SST or SSTA combined with SSTR to anti tumor effects, including direct anti proliferative effect, indirect anti-proliferative effect, promoting tumor cell apoptosis and inhibiting tumor angiogenesis, immune regulation.

Small sample studies on the SSTR subtypes expression at the surface of lymphoma cells have shown that orbital lymphoma cells (6 cases) could express SSTR2 and SSTR3, neck zone lymphoma cells (2 cases of Hodgkin's disease and 2 cases of non-Hodgkin's lymphoma) was only the expression of SSTR2. All the lymphoma cell which were studied had no SSTR1, SSTR4 and SSTR5 expression. And SSTR2 or SSTR3 expression level was relatively low [1].

Currently for lymphoma diagnosis, staging and prognostic evaluation of the main means of nuclear medicine are PET-CT, 67 Ga scintigraphy, receptor imaging, and lymphoscintigraphy. Although PET-CT is the most important nuclear medical examination for diagnosis and staging of lymphoma, its diagnostic sensitivity is closely related with the differentiation degree and different sub-type of the lymphoma, and also less reliable diagnosis of bone marrow lymphoma lesions[3-8]. In addition, due to the expensive inspection costs (large-scale clinical use is restricted) also make clinical large-scale use is restricted. Gallium-67 scintigraphy, receptor imaging and other imaging methods, there are also a variety of deficiencies. Over the last decade with the research and development of somatostatin and somatostatin receptor, somatostatin receptor imaging in lymphoma diagnosis, staging and prognostic evaluation interest more and more people to pay attention to. Although the SSTR2 or SSTR3 expression was low at the surface of lymphoma cells, the early experiments *in vitro* and clinical imaging studies have shown that somatostatin receptor scintigraphy may be a better diagnosis of lymphoma and staging, also can find a small number of focal bone marrow lymphoma (10%)[9]. Main mechanism is the radiolabeled somatostatin analogues and somatostatin receptor

binding, to achieve imaging and therapeutic effects of lymphoma or benign lesions. Studies have shown that the lymphoma cells of different parts have different diagnostic sensitivity of the imaging: the department of the neck and body surface is higher, followed by the top of the diaphragm, and pelvic lowest [10], compared with other imaging methods, it has less cost, simple and less side effects, but further enhanced sensitivity. Zhao Deshan in the study pointed out that the lower sensitivity of 99Tcm-octreotide scintigraphy to detect lymphoma lesions is not appropriate for the staging of lymphoma patients first-line method, but can be a useful addition to conventional imaging method [11].

Somatostatin analogues and radionuclide marked octreotide become complementary method of treatment for refractory or recurrent malignant lymphoma, the 99Tcm-octreotide imaging will also be an effective method of screening appropriate patients. In recent years, the application of new drugs and treatment means, such as monoclonal antibody targeted a specific antigen, the high-dose chemotherapy supported by autologous hematopoietic stem cell, radioimmunotherapy, and dendritic cells has enhanced the lymphoma therapy, but these new drugs and new means are with some deficiencies, such as expensive cost which would hinder theirwide application in clinical practice. SS and its anal ogs has an important role to play in the treatment of many tumors which express SSTR. The doxorubicin is connected to the SSA to get drug conjugates AN-162 and AN-238 could inhibit the tumor growth of lung cancer, colon cancer, glioblastoma, liver cancer in animal experiments, and reflect the low toxicity to normal tissues[12-15]. Wang Jian studies have shown that octreotide paclitaxel conjugates, including PTX-OCT, PTX-Tyr-OCT, 2PTX-OCT and 2PTX-Tyr-OCT, can significantly inhibit the growth of SCLC cell lines H446 of SSTR high expression, and the conjugates showed a low toxicity in fibroblasts without SSTR's expression [16]. On the basis of the SSTR ligand-specific binding, as well as gamma or positron radionuclide marker ligand imaging, the radionuclide marker SSTA can be the targeted therapy for tumor of high expression SSTR, peptide receptor radionuclide therapy. Currently, the most commonly used for peptide receptor radionuclide therapy radionuclide are 90Y and 177Lu. 90Y can launch pure high-energy beta-ray which suit to larger tumors and a variety o f SSTR-expressing tumor.In the rat model of subcutaneous tumor, 90Y -DOTA-TOC (90Y-DOTA-Try (3)-octretid) can effectively control tumor growth [17]. The proper high-affinity SSTA of radiolabeled will be the treatment of lymphoma, and may have a very good prospect which needs further study.

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生长抑素受体亚型 SSTR2、SSTR3 在淋巴瘤中的表达及临床意义

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摘要 目的：探讨生长抑素受体亚型 SSTR2 和 SSTR3 在不同类型、不同部位淋巴瘤中的表达情况并分析其临床意义。方法：采用 RT-PCR 法检测 105 例 4 种不同淋巴瘤石蜡标本中 SSTR2 和 SSTR3 的基因表达情况。结果：SSTR2 及 SSTR3 在粘膜相关性淋巴瘤阳性表达率分别为(8/27)，(6/27)，弥漫大 B 细胞型淋巴瘤(14/36)，(12/36)，NK/T 细胞淋巴瘤(9/22)，(6/22)，伯基特淋巴瘤(6/20)，(7/20)，SSTR2 的总阳性率为（37/105），SSTR3 的总阳性率为（31/105）。其中病变位于膈上的 SSTR2 的总阳性率为（24/105），膈下的总阳性率(14/105)，而 SSTR3 在膈上的总阳性率为（19/105），膈下的为（11/105）。结论：部分淋巴瘤组织中至少表达一种生长抑素受体，且表达率较低，但淋巴瘤是对放射性敏感的肿瘤，低表达的生长抑素受体对淋巴瘤的诊断及靶向治疗方面是否有意义，还需要进一步研究。

关键词 生长抑素；受体；淋巴瘤；RT-PCR

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