RESEARCH ARTICLE

Somatostatin Receptors 3, 4 and 5 Play Important Roles in Gallbladder Cancer

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Abstract

Expression changes of somatostatin receptor subtypes (SSTRs) including SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5 in the development of gallbladder cancer were assessed with attention to relationships with clinical pathological characteristics. SSTRs in 29 gallbladder cancer and 25 normal gallbladder tissue specimens were examined by immunohistochemical staining. Differences between SSTRs expressions and clinical pathological parameters were analyzed by chi-square test. The five subtypes of SSTR were all expressed in gallbladder cancer tissues and SSTR3 presented the highest expression. SSTR5 expression was increased significantly in gallbladder cancer ($P<0.05$) compared with that in normal gallbladder tissue. SSTR3 expression in highly and moderately differentiated gallbladder cancer was significantly higher than that in poorly differentiated lesions ($P<0.05$). SSTR4 expression was lower in gallbladder cancer with lymph node metastasis than that in gallbladder cancer without lymph node metastasis ($P<0.05$). Therfore, these results indicated that SSRT5, SSTR3 and SSTR4 may play important roles in the formation and development of gallbladder cancer.

Keywords: Gallbladder cancer - somatostatin (SST) - somatostain receptor (SSTR)

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Introduction

Gallbladder cancer, as a frequent cancer existing in alimentary canal, threatens human health these years. Gallbladder cancer is characterized by hiding onset, atypical symptoms, quick development, high mistake diagnosis rate, low prognosis and postoperative survival rate (de Aretxabala et al., 2004; Kim et al., 2012). In order to raise the survival rate of patients, early diagnosis and detection of metastasis need to be done in therapy of gallbladder cancer. Somatostatin (SST), as a kind of growth hormone releasing inhibiting hormone, is engaged in regulation of hormones secretion in different ways and formation of gallbladder cancer (Pisarek et al., 2009). SST is isolated from brain, which can inhibit pituitary releasing somatropin (Patel, 1999). SST exists in human endocrine and exocrine systems and possesses multiple biological effects. SST has two kinds of molecular forms: 14 peptide somatropin (SS214) and 28 peptide somatrpin (SS228), which have the similar structure and bioactivity including inhibition the function of endocrine and exocrine cells, regulation the movement of biliary tract and gastrointestinal tract, adjustment the recognition function of brain as neuromediator (Reubi et al., 1997; Hofsli, 2002). Moreover, SST plays an important role in regulating cell proliferation and differentiation, which inhibits the proliferation of various kinds of cell including tumor cells (Peverelli, et al., 2012). Research shows that SST and its analogs could notably inhibit the proliferation of normal cells or tumor cells in vivo and in vitro, since SST could suppress the formation of tumor vessel, block cell cycle and induce apoptosis of tumor cells via releasing hormone or cytokines which can inhibit tumor growth (Garske et al., 2012; Hashimoto, et al., 2013; Kuriyama et al., 2013).

A plenty of researches show the regulatory action of SST counts on specific receptors expression presenting on target cells (Hall et al., 2002; Pisarek et al., 2009). Somatostatin receptor (SSTR), as a G protein coupled receptor, formed by seven specific transmembrane segments and expressed in multiple human primary tumors (Hall, et al., 2002). SSTR has five subtypes: SSTR1-5. SSTR2 has two different isomers: SSTR2A and SSTR2B. SSTR2, SSTR3, SSTR5 and synthesized SSTR analogs have higher affinity for their similarity with ligands on high selectivity and functional response (Kouroumalis et al., 1998; Barbare et al., 2009). SSTR 1, SSTR2, SSTR 4, and SSTR 5 are concerned with the blockade of cell cycle and could block cell in G1/G0periond (Buscail et al., 2002; Bousquet et al., 2004). SST can adjust apoptosis via SSTR2 and SSTR3 (Ruscica et al., 2010).

An amount of research has shown that SSTR expression has an intimately relationship with multiple tumor diseases including breast cancer, pulmonary neuroendocrine tumor and uveal melanoma (Seitz et al., 2013; Treglia et al., 2013; Valsecchi et al., 2013). However, how SSTR

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Table 1. Clinicopathological Characteristics of Gallbladder Cancer Patients (n=29)

Age(years)	
<55	10
≥55	19
Gender	
Male	10
Female	19
Histological type	
Tubular and papillary adenocarcinoma	14
Mucinous and villous adenocarcinoma	13
Poorly differentiated cancer and others	2
Differentiation degree	
Low	5
Middle	15
High	19
Prognosis	
Recovery	16
Improvement	9
Death	4
Lymph node metastasis	
Positive	11
Negative	18
Invasion depth	
Full-thickness	12
Subserous layer	17
Clinical stage	
I	11
II	12
III	4
IV	2
Liver metastasis	
Positive	5
Negative	24

participates in gallbladder cancer focus less attention. Our research designed to investigate the expressions of the five SSTR subtypes in human primary gallbladder carcinoma tissue by immunohistochemisty (IHC). We suppose to find the relationship between the SSTRs and gallbladder cancer in clinical pathology characteristics, which could provide certain supports for diagnosis, therapy and prognosis of gallbladder cancer.

Materials and Methods

Patients

Twenty-nine patients with gallbladder cancer diagnosed from January 2000 to October 2011 were obtained and assessed from the general surgery department of Shanghai Jiading Hospital. All of the cases owned entire clinical pathology records and had no history of radiotherapy, chemotherapy or hormonal therapy before surgery. The 29 specimens of gallbladder cancer tissue were obtained from 29 patients (19 women and 10 men) who were diagnosed histopathologically as adenocarcinoma. Another 25 specimens of normal gallbladder tissue were obtained from 25 patients suffering cholecystitis and cholecystolithiasis (Table 1).

Immunohistochemistry (IHC)

Samples of gallbladder tissues were fixed with 10% formalin and embedded with paraffin, and then were sliced into 4μm pieces. Slices were dewaxed in xylene for 20min for three times and hydrated in alcohol in the gradient of 100%-100%-95%-95%-75% for 5 min in each gradient. Then slices were incubated in H2O2 for 5 min and washed by distilled water for 5 min. Slices were retrieved in retrieval buffer (containing 2% 0.5M Tris-HCl, 0.5M EDTA (pH 8.0), pH 7.8-8.0) for 10 min microwave treatment and cooled for 20 min. Slices were blocked in BSA (Bull Serum Albumin) for 10-15min and incubated with primary antibodies including anti-SSTR1, anti-SSTR2, anti-SSTR3 (1:100 dilution, Wuhan Boster Bio-engineering Limited Company, Wuhan, China), anti-SSTR4 and anti-SSTR5 (1:2500 dilution, Shanghai Meijin Biotechnology Company, Shanghai, China) antibodies for 1.5-2h at room temperature. This was followed by incubation with the goat anti-antibody as the secondary antibody for 30 min at room temperature. The immunoreaction was visualized with 3, 3'-diaminobenzidine (DAB) solution (Schulz et al., 1998; Pisarek et al., 2009).

IHC results assessment

The immunoreactive intensity for specific receptor proteins was scored semi-quantitatively using a descriptive scale as follows: strong staining (+++), moderate staining (++), weak staining (+) and pale staining (-). Subcellular distribution of SSTR subtypes (membranousor cytoplasmic) was also determined. The stained slices were analyzed by the same pathologist to avoid inter-observer error.

Statistical analysis

Data were analyzed by SPSS 11.0. Differences between SSTRs expression and clinical pathological parameters were analyzed by chi-square test. $P<0.05$ was considered statistically significant.

Results

Expressions of SSTRs in normal gallbladder tissue and gallbladder cancer tissue

Immunoreactivity of investigated gallbladder tissues was estimated in pathologically altered cells of gallbladder cancers and normal gallbladder tissues. Five subtypes of SSTR were all expressed in gallbladder cancer tissues and the brown-yellow particles mainly distributed in the cytoplasm and on cytomembrane (Figure 1). Our results showed that, the distribution of SSTR1-5 in gallbladder cancer tissue was 62.1%, 13.8%, 82.6%, 24.1% and 69.0%, respectively (Table 2). SSTR3 had the highest expression in gallbladder cancer, and SSTR2 presented the least expression in gallbladder cancer. The distribution of SSTR1-5 in normal gallbladder tissue was 44%, 12%, 64%, 16% and 24%, respectively (Table 2). Likewise, SSTR3 possessed the highest expression in normal gallbladder, which indicated that SSTR3 had an intimate relationship with gallbladder cancer. The expression of SSTR1 was higher than that of SSTR5 in normal gallbladder tissue, while the expressions of SSTR1 and SSTR5 presented an opposite condition in gallbladder cancer tissue. Thus, SSTR5 expression was increased in gallbladder cancer (P =0.045), suggesting that SSTR5

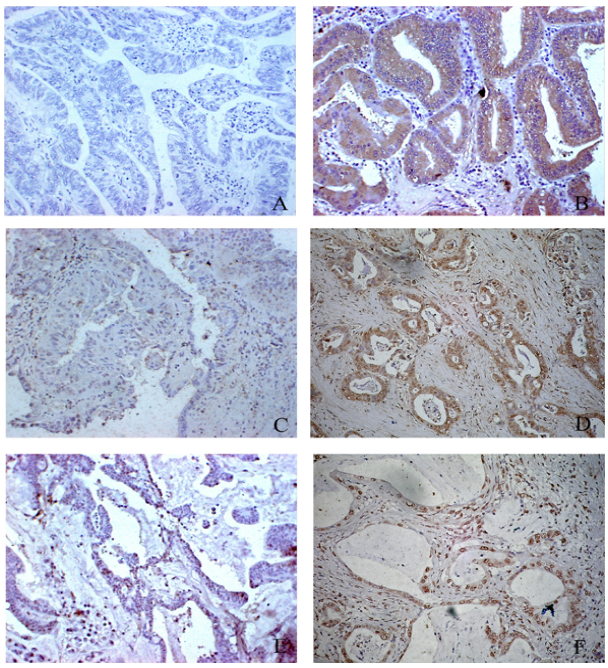

Figure 1. Expression of SSTRs in Gallbladder Cancer Tissue. The brown-yellow particles mainly distributed in the cytoplasm and on cytomembrane. A. Negative control group; B. Positive SSTR1 expression; C. Positive SSTR2 expression; D. Positive SSTR3 expression; E. Positive SSTR4 expression; F. Positive SSTR5 expression (×20)

Table 2. Expression of Somatostatin Receptor Subtypes in Gallbladder Cancer and Innormal Gallbladder Tissue of Patients Suffering Cholecystitis or Cholecystolithiasis by IHC

Gallbladder cancer tissue Subtypes of SSTR	Total positive	+++ cytopl	++ cytopl	+ cytopl
SSTR1	18(62.1%)	9	6	3
SSTR2	4(13.8%)	1	1	2
SSTR3	24(82.7%)	14	6	4
SSTR4	7(24.1%)	0	4	3
SSTR5	20(69.0%)	10	7	3
SSTR5	20(69.0%)	10	7	3

Normal gallbladder tissue Subtypes of SSTR	Total positive	+++ cytopl	++ cytopl
SSTR1	11(44%)	5	4
SSTR2	3(12%)	0	2
SSTR3	16(64%)	7	5
SSTR4	4(16%)	0	3
SSTR5	6(24%)	2	3

may affect the formation of gallbladder cancer.

Association of SSTRs expressions with the clinical pathology

As illustrated in Table 3, SSTR3 expression in poorly differentiated gallbladder cancer was significantly decreased compared with that in high and middle differentiated gallbladder cancers ($P<0.05$). SSTR3 expression was concerned with the differentiation grade of cancer and malignant degree of gallbladder. Besides, SSTR4 expression was decreased markedly in gallbladder cancer tissue with lymph node metastasis compared with

Table 3. The Relationship of SSTRs Expressions with Clinicopathological Characteristics of Gallbladder Cancer Patients

	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Age(years)					
<55	5	2	8	1	7
≥55	13	2	16	6	13
Gender					
Male	7	3	7	3	8
Female	11	1	17	4	12
Histological type					
Tubular and papillary adenocarcinoma	10	2	13	2	11
Mucinous and villous adenocarcinoma	6	2	10	4	8
Poorly differentiated cancer and others	2	0	1	1	1
Differentiation degree					
Low	1	0	0	1	2
Middle	11	3	15	5	12
High	6	1	9	1	6
Lymph node metastasis					
Positive	6	0	8	0	6
Negative	12	4	16	7	14
Invasion depth					
Full-thickness	8	1	9	2	8
Subserous layer	10	3	15	5	12
Liver metastasis					
Positive	3	1	4	1	3
Negative	15	3	20	6	17
Prognosis					
Recovery	9	2	12	5	13
Improvement	7	2	10	2	6
Death	2	0	2	0	1

that in free-lymph node metastasis gallbladder cancer tissue ($P<0.05$) suggesting that these two subtypes of SSRT may play important roles in the formation and development of gallbladder cancer. No significant differences of SSTRs expressions were shown in the age of patients, histologic type of tumor, invasion depth or liver metastasis.

Discussion

SST, a kind of regulatory peptide, was discovered in 1973 as hypothalamic hormones to inhibit growth hormone (Gottsmann et al., 1975; De et al., 1984). SST exists in human endocrine and exocrine systems and possesses general biological effects. The main function of SST is inhibiting the secretion of hypophysis hormones including pituitary growth hormone, thyroid stimulating hormone and adrenocorticotropic hormone. SST could also inhibit the secretion of multiple endocrine and exocrine cells, depress the peristalsis of biliary and gastrointestinal tracts, conduct the motion and noetic function of brain as neurotransmitter (Olias et al., 2004; Gong et al., 2010). Besides, SST suppresses the proliferation and induction of apoptosis of tumor cells (Olias et al., 2004; Quan et al., 2010). Recent research find that SST could inhibit the proliferation of gallbladder cancer cell line GBC-SD and induce the apoptosis of GBC-SD (Gong et al., 2010; Quan et al., 2010). The synthetic analogs of SST were

applied in resisting proliferation of tumor cells on clinic and received widespread attention.

SST and its synthetic analogs develop various biologic activities via SSTR on target cell membrane. Five SSTR subtypes have been identified, i.e. SSTR 1-5 (Patel et al., 1993; Hoyer et al., 1995). All of the SSTRs belong to a group of G-protein coupled receptors (Hoyer et al.,, 1995; Reisine et al., 1995) and are down-regulated by adenylatecyclase and encoded by five genes presenting on separate chromosomes (Bell et al., 1995; Toumpanakis et al., 2013). The five subtypes of SSTR possess different effects and the prolonged action of SST is mediated through the combination with SSTR2, SSTR3 and SSTR5 (Kailey et al., 2012; Moss et al., 2012). The receptor follow-up effects mediated by SSTRs are decided by the affinity of SSTRs with SST or its analogs and the density of SSTRs. The density of SSTRs positively regulates the inhibitory effects of SST and its analogs on proliferation of tumor cells. SSTR5 is related to the control of trypsin and secretion of glucagon. SSTR2 and SSTR3 induce cell apoptosis and SSTR1 and SSTR5 are related with cell cycle and affect cell proliferation and differentiation (Lamberts et al., 2002). Research has shown that SSTR5 is concerned strongly with infiltration of tumor and the expression of SSTR5 in insulinoma is lower markedly than normal tissue, indicating that SSTR5 has an intimately relationship with the proliferation of tumor (de Sa et al., 2006). Thus, therapeutic drugs effects depend on the expression of SSTRs in gallbladder cancer tissue. However, SSTRs don't express on all tumor tissue of patients suffering gallbladder cancer. Some researches indicate that the expression of different SSTRs determine the therapeutic effect of SST analogs on treatment of gallbladder cancer. Selective treatment via inhibiting relevance SSTRs expressions could improve the response rate and effect in gallbladder cancer therapy.

It is still unclear that whether SSTRs express in gallbladder cancer and the expression level of the five SSTRs. Our research designed to investigate the protein expression of SSTRs in 29 cases of primary gallbladder carcinoma patients by IHC, and found that the subtypes of SSTR expressed in different degree in gallbladder cancer tissue. SSTR3 showed the strongest expression in gallbladder cancer tissue (82.8%, Table 2). SSTR1 (62.1%) and SSTR5 (69.0%) protein expressed much either and SSTR2 protein (13.8%) had the least expression. Given that SSTR2 and SSTR3 could up-regulate cell apoptosis (Susini et al., 2006), the existence of SSTR2 and SSTR3 expressions became a vital factor that whether gallbladder cancer patients needed to accept SST or SST analogs therapy. The application of receptor imaging technology in diagnosis and therapy of gallbladder cancer should choose SSTR3 as target.

Furthermore, our results illustrated that the expression of SSTR5 in gallbladder cancer tissue had increased significantly compared with the expression in normal gallbladder (Table 2), indicating that the expression of SSTR5 could be considered as a target in gallbladder cancer diagnosis. Meanwhile, lymphatic metastasis, differentiation degree and invasion depth are considered as the mainly factor to evaluate the malignant degree of cancer. Thus, we compared the SSTRs expressions of high and middle differentiation degree with low differentiation degree in samples of gallbladder cancer patients, and found that SSTR3 expression presented positive correlation with differentiation degree (Table 3). Whereas, the expression of SSTR4 showed a negative correlation with lymphatic, indicating that SSTR3 and SSTR4 participating in the development of gallbladder cancer actively. Besides, the expression of five subtypes of SSTR showed no apparently correlating property with the age of patients, the histological types of tumor, invasion depth and liver metastasis. Agonists with high selectivity for SSTR2 or SSTR5 could suppress gallbladder emptying, whereas SSTR1-, SSTR3- and SSTR4-selective agonists failed to cause gallbladder contraction (Kaczmarek et al., 2010) and SST induced apoptosis concerning the expression of p53 and Bax protein (Songgang et al., 2009). Consider of less clinical cases in our research, further study on more cases of gallbladder cancer patients need to be carried on the mechanism of SSTR mediated gallbladder cancer and the mechanism of SSTR participates gallbladder cancer formation needs to carry out (Behr et al., 2002; Fiebiger et al., 2002; Chopra, 2004; Harada et al., 2012).

In conclusion, the inhibiting effect of SST and its analogs towards tumors via SSTR is generally acknowledged. Cell canceration is a complicated process and regulated by multiple genes and growth factors. Our research provides some insights on the clinical application of SST and its analogs to gallbladder cancer therapy.

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