The Correlations Between the Expression of SSTR2, SSTR5 Proteins and Clinicopathological Parameters as well as Prognosis of Gastric Neuroendocrine Neoplasms

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Research

Keywords: SSTR2, SSTR5, gastric neuroendocrine neoplasms, G-NENs, Prognosis

Posted Date: September 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-66797/v1

Abstract

Background Somatostatin receptor 2, 5 (SSTR2, SSTR5) were seldom investigated in gastric neuroendocrine neoplasms (G-NENs). The purpose of the study was to elucidate the expression of SSTR2, SSTR5 in G-NENs and related clinical significance.

Methods 66 paraffin-embedded specimens were obtained from The first affiliated hospital of Zhengzhou university. The expression of SSTR2 and SSTR5 was detected by immunohistochemistry. The expression of SSTR2, SSTR5 and the clinicopathological characteristics, related immunohistochemical molecules and prognosis of gastric neuroendocrine neoplasm were analyzed statistically.

Results The expression rate of SSTR2 protein in G-NENs tissues and normal stomach tissues was 48.5% and 25.0%, respectively (P=0.046); the expression rate of SSTR5 protein in G-NENs tissues and normal stomach tissues was 65.2% and 25.0% , respectively (P=0.018). The expression of SSTR2 was positively correlated with the expression of Ki-67, SSTR5 and tumor grade (P-value was 0.032, 0.002, and 0.005, respectively); the expression of SSTR5 was positively correlated with the expression of SSTR2, Ki-67, CD-56 and tumor grade (P-value was 0.032, 0.011, 0.008, 0.028, respectively). In the SSTR2-positive group, SSTR5, CD-56, Ki-67 were closely related to the prognosis of patients with G-NENs. In the SSTR5-positive group, tumor grade, SSTR2, CD-56, Ki-67 were closely related to the prognosis of patients with G-NENs. Multi-factor analysis showed that SSTR2 and SSTR5 were independent prognostic factors for patients with G-NENs.

Conclusion. High expression of SSTR2 and SSTR5 protein was related to the tumorigenesis of G-NENs. SSTR2 and SSTR5 were associated with the prognosis and might improve the prognosis of G-NENs.

Background

The Somatostatin receptor (SSTR) family with its closely five related human members (SSTR1–SSTR5) is a G protein-coupled receptor encoded by five different genes on a single chromosome and is a glycoprotein with seven transmembrane segments[1,2].The distribution of SSTRs was universal and abundant in all tissues of the human body. Somatostatin mainly exists in the central nervous system, pancreas and gastrointestinal tract, which has a wide range of inhibitory functions, including the inhibition of hypothalamic hormone, gastrin and gastric acid secretion, insulin release and so on [3-5]. It has also been shown to inhibit cell proliferation and have anti-inflammatory effects. These properties make somatostatin a potential candidate for the treatment of many diseases. Currently, it is commonly used to treat acromegaly, Cushing's disease and neuroendocrine neoplasm. The effect of somatostatin on different tissues depends on the type of somatostatin receptor (SSTR) expressed on the surface. Wang Y's study showed that SSTR was overexpressed in gastrointestinal pancreatic neuroendocrine neoplasm, especially the expression of SSTR2 and SSTR5; Positive expressions of SSTR2 and SSTR5 can predict the survival rate of patients with GEP-NENs; and the expression of various SSTRS on tumor cells constitutes the basis of somatostatin treatment for patients with neuroendocrine neoplasm [6].

At present, the expression and mechanism of SSTR subtypes in gastric neuroendocrine neoplasm have not been fully confirmed and the therapeutic effect varies greatly. It is reasonable to speculate that the poor therapeutic effect of somatostatin analogues is related to the loss of endogenous somatostatin receptor expression or the expression amount and subtype combination. So it is particularly necessary to deeply explore the relationship between SSTRs and gastric neuroendocrine neoplasm.

Therefore, the purpose of this study was to detect the expression of SSTR2 and SSTR5 in 66 G-NENs tissues with complete followup information as well as 12 normal stomach tissues by immunohistochemical staining. Combining patients' clinicopathological characteristics and follow-up data, correlation analysis and prognostic analysis were carried out to verify whether the expression of SSTR2 and SSTR5 protein had clinical significance. Furthermore, some common molecular markers of G-NENs in our cancer center, including CK-8, CD-56, CgA, Syn, and Ki-67, were detected by immunohistochemical staining and introduced to analyze their association with the expression of SSTR2 and SSTR5 protein. We expected that SSTR2 and SSTR5 might provide new ideas and methods for the diagnosis and treatment of this tumor.

Methods

Patients and Tissue samples

Paraffin-embedded tissue specimens of 66 consecutive primary G-NENs and 12 normal stomach tissues (more than 5 cm away from tumor border) were collected by the first affiliated hospital of Zhengzhou university (Zhengzhou, China). All patients were diagnosed and treated at the Department of Gastrointestinal surgery in 2015-2018. 66 G-NENs patients had not undergone any chemotherapy, radiotherapy, and other treatments before radical gastrectomy were performed. All cases were pathologically documented and personal files were recorded to obtain clinical data,under the approval of the hospital's ethical committee. SPSS 22.0 was used to collect and sort patients' clinicopathological and follow-up data, including gender, age, location of tumors, tumor size, invasion depth, lymph node status, nerve and vessel invasion, and follow-up data. The classification of gastric neuroendocrine neoplasms was based on the WHO classification and classification standards of digestive system tumors in 2010 [7]. Follow-up data were obtained by phone, letter, and the outpatient clinical database. Survival time was calculated from the date when surgery was done to the date of death or the date of the last followup. The follow-up period was closed in march 31, 2020. A followup of all patients was carried out according to our standard protocol (every 3 months for at least 2 years, every 6 months for the next 3 years, and after 5 years every 12 months for life). Median follow-up in patients still alive at analysis was 36.5 months (range, 12-54 months).

Immunohistochemical Staining

SSTR2, SSTR5, CK-8, CD-56, CgA, Syn, and Ki-67 expression in postoperative paraffin-embedded tumor specimens of all selected patients were detected by immunohistochemical method. The concentration of antibodies and positive site were as follows: anti-SSTR2, dilution 1:100, positive site is cytoplasm or membrane; anti-SSTR5, dilution 1:50, positive site is cytoplasm or membrane; anti-CK-8, dilution1:100, positive site is cytoplasm; anti-CD-56, dilution 1:50, positive site is membrane; Anti-CgA, dilution 1:50, positive site is cytoplasm; anti-Syn, dilution 1:50, positive site is cytoplasm; anti-Ki-67, dilution 1:100, and positive site is nucleus. The detailed staining procedures were strictly followed the supplier's recommendation. Negative controls were obtained by incubation of parallel slides omitting the primary antibodies. Besides, sections known to be stained positively in each run were served as positive controls.

Immunohistochemical Staining Scoring

Slides were all semi-quantitatively evaluated by two independent pathologists (L. W.C.and H.H.Y), who were blinded to patients' clinical data when scoring immunohistochemical results of the archival tissue samples. The initial score was divided into four levels by the range of cell staining: -, no cell staining or range <10%; +, 11-25% staining; ++, 26 – 50% staining; +++, 50% > staining. The expressions of SSTR2 and SSTR5 are mainly cytoplasmic staining. By semi-quantitative analysis, the immunostaining of SSTR2 and SSTR5 can be divided into four grades, negative (-), low (+), intermediate (++), and high expression (+++). When the proportion of Ki-67 positive cells was calculated, 500-2000 cells were counted in the most strongly labeled and stained area under at least 50 microscope high-power field (1 high-power field =2mm), and the proportion of Ki-67 positive cells in tumor cells (i.e., Ki-67 index) was calculated. In the final statistical analysis, the negative staining of CK-8, CD-56, CgA, and Syn was defined as the tumor cytoplasm staining ≤10%. Positive staining of CK-8, CD-56, CgA and Syn was defined as >10% staining of tumor cytoplasm or cell membrane. The above diagnostic criteria refer to the product specification and related literature of zhongshan jinqiao company.

Statistical Analysis

The association between SSTR2, SSTR5 expression and clinicopathological factors as well as other immunohistochemical markers was evaluated by chi-square test. The 3-year survival rate was calculated by the Life Tables method, survival curves were obtained by Kaplan-Meier method and differences between survival curves were examined with the log-rank test. All the statistically significant variables observed in univariate analysis were investigated by means of multivariate analysis using the Cox proportional hazards model. All statistical tests were two-sided, and significance was set at the 0.05 level. Statistical analysis and graphics were performed by the SPSS 22.0 statistical software package (SPSS, Chicago, IL).

Results

Clinicopathological Characteristic of Gastric neuroendocrine neoplasms Patients

66 G-NENs patients have been recruited in this study, 50 males and 16 females; 36 patients with ≤60 years while 30 patients with more than 60 years at the data of diagnosis; 47 patients located in the cardia and body of gastric and 19 patients located in the gastric antrum; the tumor size of 23 patients were <4cm while 43 patients had tumor size ≥4cm; 10 patients with tumors invaded the mucosa and submucosa, 12 patients with tumors invaded the muscularis and 39 cases patients with tumors invaded the serosa; 22 cases without lymph node metastasis while 40 cases with it; patients with or without vessel and nerve invasion were 28 and 15 cases, respectively; furthermore, 10 patients with G1, 16 patients with G2, 8 patients with G3 NEN and 32 patients with G3.

Expression of Markers in Gastric neuroendocrine neoplasms

66 paraffifin-embedded G-NENs tissues and 12 normal stomach specimens were all selected from the Department of pathology, the first affiliated hospital of Zhengzhou university in 2019.

SSTR2 expression in G-NENs tissues were as follows: - , 34 cases (51.5%); +, 10 cases (15.2%); ++, 15 cases (22.7%); +++, 7 cases (10.6%), respectively. SSTR2 expression in normal stomach tissues from - to +++ were 9 cases (75.0%), 0 cases (0%), 3 case (25.0%), and 0 case (0%), respectively. Therefore, the positive expression of SSTR2 in G-NENs tissue and normal stomach tissue were 48.5% and 25.0%, respectively. Pearson χ^2 test showed that SSTR2 expression in two groups had significant different (χ^2 value=4.00, P =0.046). In other word, positive expression of SSTR2 protein existed in G-NENs tissue. Figure 1a and 1b illustrated the positive expression and negative of SSTR2 in the cytoplasm of G-NENs cells, respectively; Figure 1c and 1d showed that the positive expression and negative of SSTR2 in the cytoplasm of normal stomach tissue cells, respectively.

SSTR5 expression in G-NENs tissues were as follows: -, 23 cases (34.8%); +, 13 cases (19.7%); ++, 22 cases (33.3%); +++, 8 cases (12.2%), respectively. SSTR5 expression in normal stomach tissues from - to +++ were 9 cases (75.0%), 1 cases (8.3%), 2 case (16.7%), and 0 case (0%), respectively. Therefore, the positive expression of SSTR5 in G-NENs tissue and normal stomach tissue were 65.2% and 25.0%, respectively. Pearson χ^2 test showed that SSTR5 expression in two groups had significant different (χ^2 value=5.60, P =0.018). In other word, the positive expression of SSTR5 protein existed in G-NENs tissue. Figure 1e and 1f illustrated the positive expression and negative of SSTR5 in the cytoplasm of G-NENs cells, respectively; Figure 1g and 1h showed the positive expression and negative of SSTR5 in the cytoplasm of normal stomach tissue cells, respectively.
Furthermore, just as Table 1 displayed, the positive rate of CK-8, CD-56, CgA and Syn expression in G-NENs tissues were 82.0% (50/61), 75.8%(50/66), 65.9% (29/44) and 97.0% (64/66), respectively. The expression of Ki-67 was more than 20% in 60.6% (40/66) G-NENs tissues.

Table 1
Correlation Between SSTR2 Expression and Clinicopathologic Characteristic of Gastric neuroendocrine neoplasms Patients

Variable	**n**	Positive group[a]	**Negative group[b]**	***P-value***
ALL cases	66	32	34	
Age(years)				0.208
≤60	36	20	16	
>60	30	12	18	
Gender				
Male	50	23	27	0.475
Female	16	9	7	
Tumor site				0.668
Cardia and body of gastric	47	22	25	
Gastric antrum	19	10	9	
Tumor size				0.141
4cm	23	14	9	-
≥4cm	43	18	25	
Invasion depth				0.213
Mucosa and submucosa	10	5	5	
Muscularis	12	3	9	
Serosa	39	21	18	
Lymph node metastasis				0.160
Yes	40	22	18	
No	22	8	14	
Vessel and nerve invasion				0.284
Yes	28	12	16	
No	15	9	6	
Tumor grade				**0.005**
G1	10	6	4	

G2	16	13	3	
G3 NEN	8	4	4	
G3	32	9	23	
CK-8				0.580
Negative	11	5	6	
Positive	50	25	25	
CD-56				0.113
Negative	16	5	11	
Positive	50	27	23	
CgA				0.750
Negative	15	8	7	
Positive	29	14	15	
Syn				0.965
Negative	2	1	1	
Positive	64	31	33	
Ki-67（%）				**0.002**
＜20	26	19	7	
20-50	10	5	5	
＞50	30	8	22	
SSTR5				**0.032**
Negative	23	7	16	
Positive	43	25	18	

The bold values in Table showed the differences in some variables were statistically significant.

Positive group[a]: +, ++, +++

Negative group[b]: -

Correlations Between SSTR2, SSTR5 Expression and Clinicopathological Parameters

To explore the association of SSTR2, SSTR5 expression and clinicopathological characteristics, 66 immunohistochemical slides of G-NENs tissues were divided into two groups for analysis, a negative group (including -) and a positive group of SSTR2and SSTR5 (including +, ++, +++).

Just as Table 1 revealed, there were no significant differences between the positive group and the negative group of SSTR2 involving in some clinicopathological parameters and molecular markers, including age, gender, tumor location, tumor size, invasion depth, lymph node metastasis, vessel and nerve invasion as well as the expression of CK-8, CD-56, CgA and Syn ($P > 0.05$). There were statistically significant differences in tumor grade, Ki-67 and SSTR5 in the SSTR2 expression group ($P=0.005$, 0.002 and 0.032, respectively).

Just as Table 2 revealed, there were no significant differences between the positive group and the negative group of SSTR5 involving in some clinicopathological parameters and molecular markers, including age, gender, tumor location, tumor size, invasion depth, lymph node metastasis, vessel and nerve invasion as well as expression of CK-8, CgA and Syn ($P >0.05$). There were statistically significant differences in tumor grade, CD-56, Ki-67 and SSTR2 in the SSTR5 expression group ($P=0.028$, 0.008, 0.011 and 0.032, respectively).

Table 2
Correlation Between SSTR5 Expression and Clinicopathologic Characteristic of Gastric neuroendocrine neoplasms Patients

Variable	**n**	Positive group[a]	**Negative group[b]**	***P-value***
ALL cases	66	43	23	
Age(years)				0.187
≤60	36	26	10	
>60	30	17	13	
Gender				
Male	50	33	17	0.798
Female	16	10	6	
Tumor site				0.829
Cardia and body of gastric	47	31	16	
Gastric antrum	19	12	7	
Tumor size				0.593
4cm	23	14	9	-
≥4cm	43	29	14	
Invasion depth				0.837
Mucosa and submucosa	10	6	4	
Muscularis	12	7	5	
Serosa	39	26	13	
Lymph node metastasis				0.508
Yes	40	27	13	
No	22	13	9	
Vessel and nerve invasion				0.606
Yes	28	19	9	
No	15	9	6	
Tumor grade				**0.028**
G1	10	7	3	

G2	16	15	1	
G3 NEN	8	5	3	
G3	32	16	16	
CK-8				0.584
Negative	11	6	5	
Positive	50	33	17	
CD-56				**0.008**
Negative	16	6	10	
Positive	50	37	13	
CgA				0.274
Negative	15	9	6	
Positive	29	22	7	
Syn				0.648
Negative	2	1	1	
Positive	64	42	22	
Ki-67（%）				**0.011**
＜20	26	22	4	
20-50	10	7	3	
＞50	30	14	16	
SSTR2				**0.032**
Negative	34	18	16	
Positive	32	25	7	
The bold values in Table showed the differences in some variables were statistically significant.				
Positive group[a]: +, ++, +++				
Negative group[b]: -				

Prognostic Analysis of Patients with Gastric neuroendocrine neoplasms in the Positive and Negative Groups of SSTR2 and SSTR5

To clarify whether the expression of SSTR2 and SSTR5 protein influenced the prognosis of G-NENs patients, combining follow-up data, survival discrepancy in the high and low expression groups of SSTR5 were revealed by Kaplan-Meier method and log rank test (Fig. 2 and Table 3, 4). Furthermore, life table method was used to calculate the 3-year survival rate.

Table 3
Survival Analysis Between Positive and Negative Group of SSTR2 in Gastric neuroendocrine neoplasms by Kaplan–Meier Method (Log-Rank Test)

Variable	Positive group[a]			**Negativegroup**[b]		
	n	**3-year survival rate**	***P-value***	**n**	**3-year survival rate**	***P-value***
Age(years)			0.262			0.078
≤60	20	85		16	75	
>60	12	91		18	50	
Gender			0.121			0.496
Male	23	91		27	56	
Female	9	78		7	71	
Tumor site			0.770			0.862
Cardia and body of gastric	22	82		25	60	
Gastric antrum	10	90		9	55	
Invasion depth			0.130			0.052
Mucosa and submucosa	5	64		5	100	
Muscularis	3	67		9	78	
Serosa	21	36		18	33	
Lymph node metastasis			0.976			0.654
Yes	22	86		18	56	
No	8	88		14	64	
Vessel and nerve invasion			0.319			0.104
Yes	9	89		16	75	
No	12	100		6	67	
Tumor grade			0.079			0.531
G1	6	83		4	75	
G2	13	92		3	67	
G3 NEN	4	75		4	50	

G3	9	89		23	57	
SSTR5			**0.000**			**0.003**
Negative	7	57		16	63	
Positive	25	96		18	67	
CK-8			0.639			0.912
Negative	5	80		6	67	
Positive	25	88		25	56	
CD-56			**0.000**			**0.029**
Negative	5	60		11	36	
Positive	27	93		19	70	
CgA			0.606			0.943
Negative	8	88		7	71	
Positive	14	86		15	60	
Syn			0.744			0.352
Negative	1	100		1	76	
Positive	21	62		33	64	
Ki-67（%）			**0.01**			0.287
＜20	19	75		7	71	
20-50	5	57		5	60	
＞50	8	54		22	55	
G3	9	89		23	57	**0.037**
SSTR2 Negative/Positive	32	82		34	35	**0.002**

The bold values in Table showed the differences in some variables were statistically significant.

Positive group[a]: +, ++, +++

Negative group[b]: -

Table 4
Survival Analysis Between Positive and Negative Group of SSTR5 in Gastric neuroendocrine neoplasms by Kaplan–Meier Method (Log-Rank Test)

Variable	Positive group[a]			**Negative group[b]**		
	n	**3-year survival rate**	***P-value***	**n**	**3-year survival rate**	***P-value***
Age(years)			0.885			0.187
≤60	26	85		10	70	
>60	17	71		13	54	
Gender			0.208			0.084
Male	33	82		17	53	
Female	10	78		6	83	
Tumor site			0.663			0.959
Cardia and body of gastric	31	77		16	56	
Gastric antrum	12	83		7	71	
Invasion depth			0.461			0.052
Mucosa and submucosa	6	100		4	75	
Muscularis	7	71		5	60	
Serosa	26	73		13	54	
Lymph node metastasis			0.797			0.462
Yes	27	81		13	54	
No	13	85		9	67	
Vessel and nerve invasion			0.802			0.174
Yes	19	89		9	78	
No	9	89		6	33	
Tumor grade			**0.012**			0.536
G1	6	86		3	67	
G2	15	93		1	0	
G3 NEN	5	60		3	67	

G3	16	56		16	63	
SSTR2			**0.002**			0.110
Negative	7	57		16	63	
Positive	25	96		18	67	
CK-8			0.856			0.889
Negative	6	83		5	60	
Positive	33	84		17	59	
CD-56			**0.000**			0.923
Negative	6	17		10	60	
Positive	37	89		13	62	
CgA			0.082			0.229
Negative	9	100		6	50	
Positive	22	77		7	86	
Syn			0.622			0.244
Negative	1	100		1	0	
Positive	42	62		22	59	
Ki-67（%）			**0.001**			0.767
＜20	22	91		4	50	
20-50	5	71		3	67	
＞50	14	64		10	63	
G3	16	56		16	63	**0.001**
SSTR5	32	83		34	76	**0.000**

Negative/Positive

The bold values in Table showed the differences in some variables were statistically significant.

Positive group[a]: +,++,+++

Negative group[b]: -

In the SSTR2-positive group, the 3-year survival rate of SSTR5-positive group was 96%, which was significantly higher than that of SSTR5-negative group (57%) and the difference was statistically significant (Fig. 2a, P=0.000). The 3-year survival rates for the CD-56 positive group and negative group

were 93% and 60%, respectively (Fig. 2b, P=0.000). Moreover, the 3-year survival rates of G-NENs patients with Ki-67 < 20%, 20%-50%, and > 50% were 75%, 57%, and 54%, respectively (Fig. 2c, P=0.01). In the SSTR2-negative group, the 3-year survival rates of SSTR5-positive and SSTR5-negative were 63% and 67%, respectively (Fig. 2d, P=0.003). The 3-year survival rate of CD-56 negative group was significantly lower than that of CD-56 positive group (36% vs. 70%, Fig. 2e, P=0.029). Meanwhile, a subgroup analysis of G3 patients in the tumor grade showed that the 3-year survival rate of G-NENs in the SSTR2-positive group was significantly higher than that in the SSTR2-negative group (89% vs. 57%, Fig. 2g, P=0.037). Moreover, the 3-year survival rate of the SSTR2-positive group increased by 47% (82% vs. 35%) compared with the SSTR2-negative group, which was statistically significant (Fig. 2f, P=0.002).

In the SSTR5-positive group, the 3-year survival rates of patients with tumor grades of G1, G2, G3 NEN and G3 were 86%, 93%, 60% and 56%, respectively (Fig. 2h, P=0.012). The 3-year survival rates for SSTR2 negative and positive patients were 57% and 96%, respectively (Fig. 2i, P=0.002). The 3-year survival rate of CD-56 negative patients was significantly lower than that of CD-56 positive patients (17% vs. 89%, Fig. 2j, P=0.000). Moreover, the 3-year survival rates of G-NENs patients with Ki-67 < 20%, 20%-50% and > 50% were 91%, 71% and 64%, respectively(Fig. 2k, P=0.001), with statistically significant differences. Meanwhile, a subgroup analysis of G3 patients in the tumor grade showed that the 3-year survival rate of G-NENs in the SSTR5-positive group was slightly lower than that in the negative group (56% vs. 63%, Fig. 2m, P=0.001). Moreover, the 3-year survival rate of the SSTR5 negative group was 7% lower than that of the SSTR5 positive group(76% vs. 83%), and the difference was statistically significant (Fig. 2l, P=0.000).

Multivariate Analysis

Cox proportional risk model was used to analyze all the patients in this study (Table 5). The results showed that SSTR2 and SSTR5 expression were independent prognostic factors for patients with gastric neuroendocrine neoplasms. CD-56 was an independent prognostic factor for gastric neuroendocrine neoplasms patients in the SSTR2 positive expression group, the SSTR2 negative group and the positive SSTR5 group.

Table 5
Independent Prognostic Factors at Multivariate Analysis by Cox Model in Gastric neuroendocrine neoplasms

Variable		X^2 value	P-value	HR	95% CI
All cases	Tumor grade	1.013	0.603	0.428	0.081–2.244
	SSTR2	7.307	0.007	5.325	1.584–17.905
	SSTR5	11.097	0.001	9.899	4.34–25.986
	Ki-67	2.755	0.252	0.368	0.113–1.198
SSTR2 Positive group	SSTR5	0.005	0.945	0.285	0.032–2.588
	CD-56	16.325	0.000	11.593	3.532–18.058
	Ki-67	5.402	0.067	0.221	0.06–0.813
SSTR2 negative group	SSTR5	0.01	0.919	0.338	0.037–3.07
	CD-56	3.536	0.06	2.838	0.957–8.418
SSTR5 Positive group	Tumor grade	0.932	0.628	0.551	0.077–1.23
	SSTR2	2.776	0.096	4.179	0.777–2276
	CD-56	12.215	0.000	15.232	3.307–70.153
	Ki-67	5.748	0.056	0.195	0.051–0.742

Discussion

Somatostatin receptor (SSTR) is a glycoprotein with 7 transmembrane segments, which mainly exists in the central system and secretory organs and is mostly related to neuroendocrine neoplasms. Currently, the recognized molecular subtype is SSTR1-5. Since SSTRs is widely used in neuroendocrine tumors, antitumor therapy is commonly used. It was found that SSTR2 was the most commonly express subtype, followed by SSTR5 [8,9]. However, there are few studies on SSTR2 and SSTR5 in gastric neuroendocrine neoplasms (G-NENs). Currently, there are no major case studies on the expression of SSTR2 and SSTR5 in gastric neuroendocrine neoplasms, and the prognostic value of SSTR2 and SSTR5 in patients with gastric neuroendocrine neoplasms has not been clearly reported. Therefore, in this study, the expression levels of SSTR2 and SSTR5 proteins were detected by immunohistochemical S-P method in the tissue samples of 66 patients with gastric neuroendocrine neoplasms and 12 samples of paracancer normal tissues. Then, correlation analysis and survival analysis were conducted based on the clinicopathological data and follow-up data of the patients.

The results of this study showed that the positive expression rate of SSTR2 in gastric neuroendocrine neoplasms tissue specimens and paracancer normal tissue specimens was 48.5% and 25.0%, respectively, with statistically significant differences. That is, the positive expression rate of SSTR2

protein in gastric neuroendocrine neoplasms tissues is higher than that in normal tissues, which is consistent with other common neuroendocrine neoplasms tissues reported in the literature. The positive expression rates of SSTR5 in gastric neuroendocrine neoplasms and paracancer normal tissues were 65.2% and 25.0%, respectively. That is, the positive expression rate of SSTR5 protein in gastric neuroendocrine neoplasms tissues is higher than that in normal tissues.

Cytokeratin (CK) is the main skeletal protein of cells. Studies have shown that CK-8, a member of the cytokeratin (CK) family, is a receptor of plasminogen, which can activate plasminogen and has the effect of reducing the extracellular matrix and basement membrane of tumor. CK-8 is closely related to the metastasis and invasion of tumor. As a member of the superimmune protein family, CD-56 molecule is an isomer of the adhesion molecules of nerve cells and a marker of natural killer lymphocytes (NK). It mediates the interaction between cells and cells, and is mostly associated with acute myeloid leukemia.Studies have also shown that CD-56 has different prognostic significance in AML of different subtypes. CgA is an acidic protein composed of 439 amino acids, which is mostly located in the dense core granules of neuroendocrine cells and is often used for the detection of neuroendocrine cells [10]. Syn is a membrane protein closely related to the function of cell transmission synapses, which is mostly located in nerve tissues [11]. Ki-67 is a nuclear antigen related to cell proliferation, which is mainly used to evaluate the proliferation activity of various tumor cells. It is currently recognized as the most optimal and convenient biological marker for tumor cell proliferation [12,13]. Ki-67 protein is highly correlated with the development, metastasis and prognosis of malignant tumors, which is an indicator closely related to cell proliferation. In addition, high tumor proliferation is usually associated with tumor invasion and metastasis. So high expression of Ki-67 often indicates poor prognosis of patients with tumor.

In this study, the expression rates of CK-8 and CD-56 were both over 60%, suggesting that gastric neuroendocrine neoplasms have the possibility of highly malignant transformation.The expression rates of CgA and Syn were 65.9% and 97.0%, respectively, which also confirmed the high specificity of CgA and Syn in the detection of gastric neuroendocrine neoplasms. Ki-67 is expressed to different degrees in gastric neuroendocrine neoplasms, which indicates that different G-NENs have different proliferation activities and prognosis. The higher the expression of Ki-67, the worse the prognosis.In order to analyze the clinical significance and prognostic value of SSTR2 and SSTR5 on gastric neuroendocrine neoplasms, this study was divided into SSTR2, SSTR5 positive group and SSTR5 negative group. Immunohistochemical results showed that SSTR2 expression was not significantly correlated with age, gender, tumor site, tumor size, invasion depth, lymph node metastasis, vessel and nerve invasion in G-NENs patients, which was consistent with relevant literature reports. SSTR2 expression was closely related to tumor grade, SSTR5 and ki-67 in G-NENs patients, and the difference was statistically significant. Moreover, SSTR2 was negatively associated with the increase of tumor grade and ki-67 while it was positively correlated with the expression of SSTR5. SSTR5 expression was closely related to tumor grade, CD-56, Ki-67 and SSTR2 in G-NENs patients, and the difference was statistically significant. Moreover, SSTR5 is negatively associated with the increase of tumor grade and ki-67 while it was positively correlated with the expression of SSTR2.

These results indicate that during the whole development process of gastric neuroendocrine neoplasms, the expression levels of SSTR2 and SSTR5 gradually decrease and the inhibitory effect on tumor proliferation is reduced, which is in favor of the growth of gastric neuroendocrine neoplasms. Moreover, the expressions of SSTR2 and SSTR5 complement each other, which are positively correlated. However, in the SSTR5-positive group, the positive rate of CD-56 increased significantly, suggesting that patients with SSTR5-positive expression of gastric neuroendocrine neoplasms have a poor prognosis. It seems to contradict the tumor grade and the expression of Ki-67 in the SSTR5-positive group. But the carcinogenesis of gastric neuroendocrine neoplasms is a complex and dynamic process with the participation of many molecules, which mutual coordinated and restrained each other. Moreover, it is important to note that in this study, tumor grade is divided into four grades (G1, G2, G3 NEN, and G3), which some scholars believe that the G3 neuroendocrine neoplasms is not a simple neuroendocrine carcinoma, but high proliferation activity of the neuroendocrine tumor and G3 neuroendocrine carcinoma [14]. The research also proved the importance of tumor grade in gastric neuroendocrine neoplasms and rationality.

Vesterinen T et al used immunohistochemistry to detect the expression of SSTR1-5 in lung neuroendocrine tumor specimens and found that the expressions of SSTR2 and SSTR5 highly expressed in lung neuroendocrine tumor, and SSTR2 expression was higher than SSTR5 expression. Survival analysis showed that SSTR2 and SSTR5 were related to their prognosis and SSTR2 was an independent prognostic factor in patients with pulmonary neuroendocrine tumor and improved the prognosis [15]. Lodewijk L et al showed that SSTR2 was related to the prognosis of patients with medullary thyroid cancer [16]. Orlova KV et al revealed that the expression of SSTR2 in merkel cell carcinoma was associated with prognosis [17]. Kiviniemi A showed that SSTR2 is associated with oligodendroglioma in glioma cells and has A good prognosis [18]. Wang Y's study showed that SSTR2 and SSTR5 are the most common SSTR2 and SSTR5 positive expressions in gastrointestinal pancreatic neuroendocrine tumors while SSTR2 and SSTR5 positive expressions can predict the survival rate of patients with GEP-NENs. Meanwhile, the expression of various SSTRS on tumor cells constitutes the basis for the treatment of patients with neuroendocrine tumors with somatostatin.

Therefore, the authors suggest that the expression of SSTR2 and SSTR5 may be a potential indicator of the prognosis of gastric neuroendocrine neoplasms. According to the above literature, SSTR2 and 5 have higher positive expression rates in various neuroendocrine tumors than those in normal tissues , which mostly believe that the expression of SSTR2 is related to the prognosis of patients and improves the prognosis. However, there are few reports on the correlation between SSTR5 and the prognosis of neuroendocrine tumors. In this study, the 3-year survival rate of positive group of SSTR2 and SSTR 5 were 47% (82% vs. 35%) and 7% (83% vs. 76%) , which were higher than those of negative group, respectively, with statistically significant differences. Single factor analysis showed that in the SSTR2 positive group, SSTR5, CD-56 and Ki-67 were closely related to the prognosis of patients with G-NENs. In the SSTR2 negative group, SSTR5 and CD-56 were closely related to the prognosis of patients with G-NENs. In the SSTR5-positive group, tumor grade, SSTR2, CD-56, Ki-67 were closely related to the prognosis of patients

with G-NENs. All of these suggest that SSTR2 and SSTR5 proteins may have a certain influence on the carcinogenesis and prognosis of gastric neuroendocrine neoplasms.

Cox multivariate analysis showed that among all patients with gastric neuroendocrine neoplasms, SSTR2 and SSTR5 were independent prognostic factors for patients with gastric neuroendocrine neoplasms, and their positive expression suggested improved prognosis, which was consistent with the study results of Vesterinen T et al in lung carcinoid. Meanwhile, CD-56 was an independent factor affecting the prognosis of G-NENs patients in the SSTR2 positive group, SSTR2 negative group and SSTR5 positive group, which suggests that SSTR2 and SSTR5 are closely related to the expression of CD-56. Although CD-56 in patients with G-NENs is rarely studied, the expression of CD-56 in acute myeloid leukemia is more studied, which belongs to the member of the super-immune protein family and has a cellular regulatory effect, indicating poor prognosis of AML [19,20]. These also indirectly reflected the positive association between SSTR2, SSTR5 expression and tumor prognosis.

Conclusion

In summary, tumor development is a complex process involving in multiple factors and many genes.This study shows that SSTR2 and SSTR5 are closely related to the development and prognosis of G-NENs, which suggest improvement of prognosis. Somatostatin and its analogues are widely used in the diagnosis and treatment of neuroendocrine neoplasms. Therefore, the study on the expression and prognostic value of somatostatin subtypes (SSTR2, SSTR5) in G-NENs is helpful for the combination of somatostatin and its analogs with traditional tumor therapy, which provide reliable evidence and promising prospects for tumor diagnosis and treatment.

Declarations

Acknowledgments

This study was supported by Department of Gastrointestinal Surgery and Institute of Clinical Medicine, The First Affiliated Hospital, Zhengzhou University and National Natural Science Foundation of China, Grant No. 81201955. Furthermore, this work was supported by the Foundation of Henan Educational Committee (Grant No.19A320080).

Authors’ contributions

Yanwei Ye supervised the study. Yanwei Ye, Chuangfeng Xiao and Yingze Li contributed to

the study concept and design. Chuangfeng Xiao. Jingjing Li Yiming Shan and Yingze Li performed experiments and/or acquired data. Chao Han and Wencai Li contributed to the acquisition of samples and clinical data. Yanwei Ye and Chuangfeng Xiao drafted the manuscript. All authors critically reviewed the manuscript for important intellectual content and approved its final version.

Funding

The Natural Science Foundation of China (Grant No.81201955) to Yanwei Ye and Foundation of Henan Educational Committee (Grant No.19A320080) to Yanwei Ye.

Availability of data and materials

All data generated or analyzed during this study are included in this published Article.

Ethics approval and consent to participate

Informed consent was obtained from all subjects and the study received the approval of the Ethics Committees of the First Affiliated Hospital of Zhengzhou University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

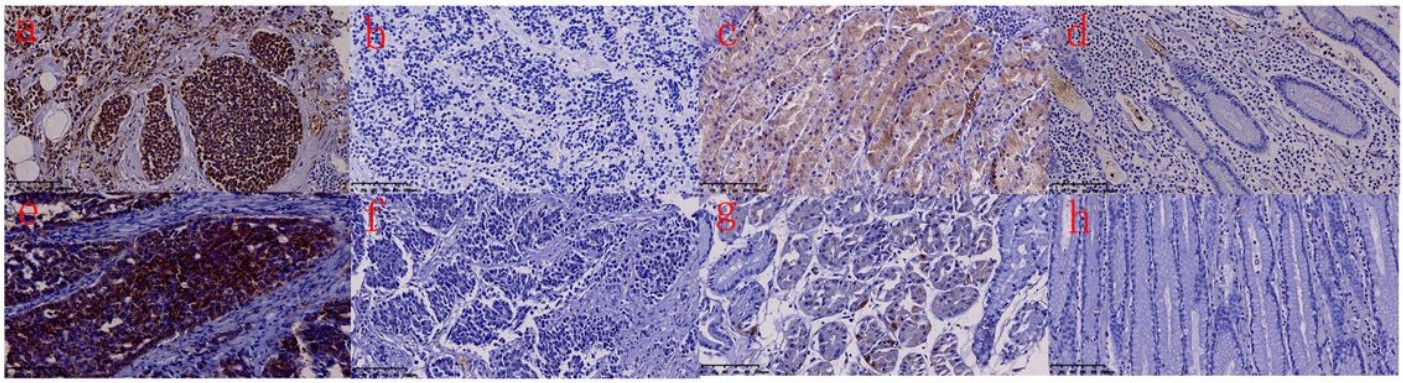

Figure 1

a-d shows immunohistochemical sections of SSTR2 expression in gastric neuroendocrine neoplasm and paracancer tissues . All immunohistochemical pictures were amplifified 200-fold. a positive expression of SSTR2 in gastric neuroendocrine tissue. b negative expression of SSTR2 in gastric neuroendocrine tissue. c positive expression of SSTR2 in paracancer normal tissue. d negative expression of SSTR2 in paracancer normal tissue. Fig. 1 e-h shows immunohistochemical sections of SSTR5 expression in gastric neuroendocrine neoplasm and paracancer tissues. e positive expression of SSTR5 in gastric neuroendocrine tissues. f negative expression of SSTR5 in gastric neuroendocrine tissues. g positive expression of SSTR5 in para-cancer normal tissues. h negative expression of SSTR5 in paracancer normal tissues.

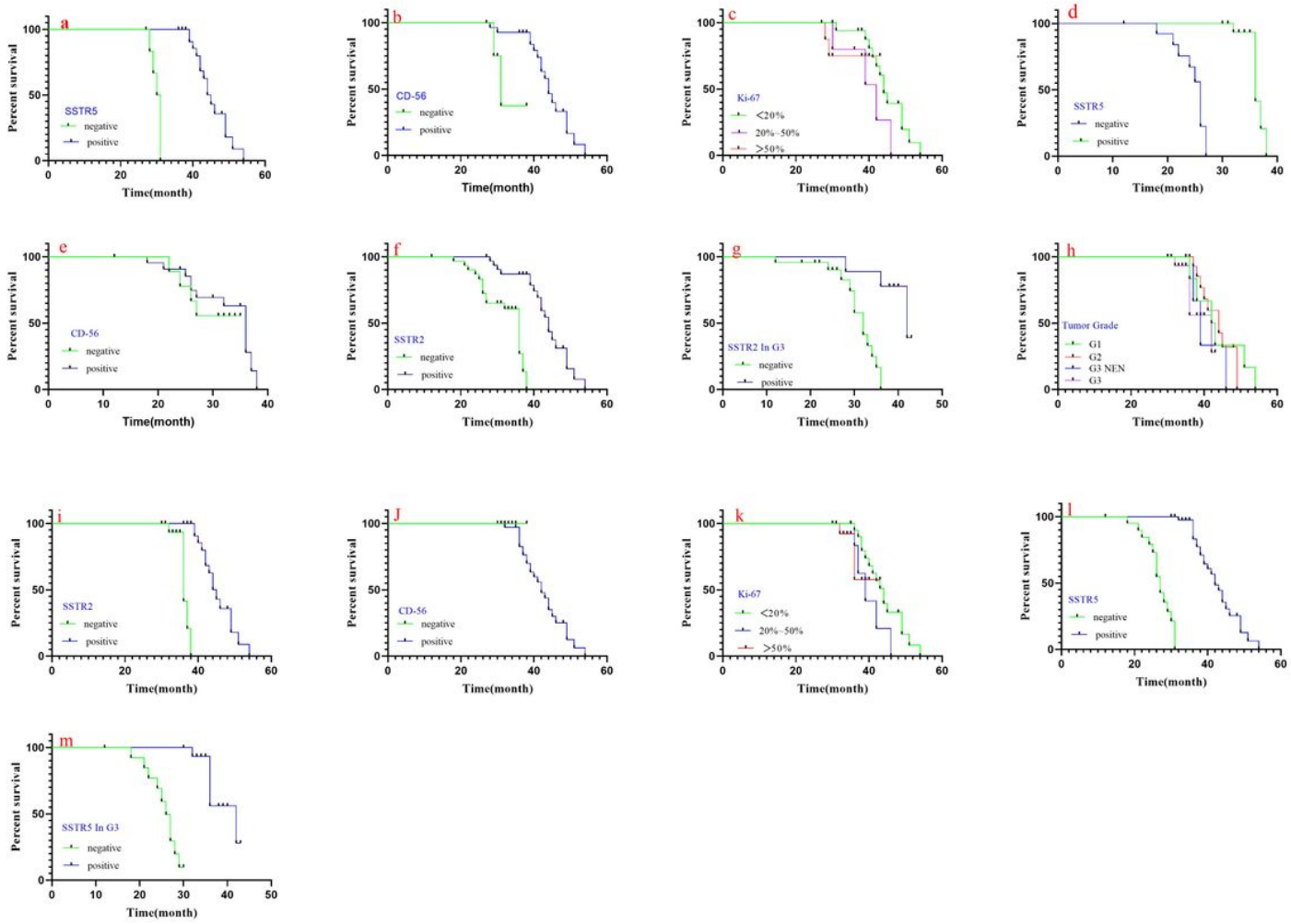

Figure 2

The survival curves of the patients with gastric neuroendocrine neoplasm by Kaplan–Meier method (log rank test). In the SSTR2-positive group, there were signifificant differences in SSTR5 expression (2a, P=0.000), CD-56 expression (2b, P=0.000), Ki-67 expression (2c, P=0.01). In the SSTR2-negative group, significant differences existed in SSTR5 expression (2d, P=0.003), CD-56 expression (2e, P=0.029). The survival difference was showed between the SSTR2-positive group and the SSTR2-negative group (2f, P=0.002).The survival difference was revealed between SSTR2-positive and SSTR2-negative gastric neuroendocrine tumor in the tumor grade G3 subgroup (2g, P=0.037). In the SSTR5-positive group, there were signifificant differences in tumor grades (2h, P=0.012), SSTR2 expression (2i, P=0.002), CD-56 expression (2j, P=0.000), Ki-67 expression (2k, P=0.001).The survival difference was showed between SSTR5-positive and SSTR5-negative in the tumor grade G3 subgroup (2m, P=0.001).The survival difference was showed between the SSTR5-positive and SSTR5-negative group (2l, P=0.000) .