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Original Article

Expression of Shh signaling pathway factors in gastrointestinal stromal tumor tissues and their associations with clinical pathological factors

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Abstract: Objective: To explore the expression of Shh signaling pathway factors in Gastrointestinal stromal tumor (GIST) tissues and their associations with clinical pathological factors. Methods: 129 GIST patient cases that underwent surgery treatment at the first affiliated hospital of Anhui Medical University from January 2001 to December 2008 and with complete records were collected. The expression of Shh signaling pathway proteins Shh, Ptch, Smo and Gli-1 was assayed using immunohistochemistry methods, and the relation between these protein expression and the tumor site, diameter, mitotic figures, NIH risk classification, reoccurrence and prognosis of GIST was analyzed. Results: The expression rate of these four proteins, Shh, Ptch, Smo and Gli-1, in GIST tissues was 90.5%, 83.3%, 85.7%, and 85.7% respectively; The expression level of Ptch was positively associated with that of Smo, but not with Gli-1; the expression level of Smo was positively associated with that of Gli-1; the expression level of Shh, Ptch, Smo or Gli-1 was not associated with patients' gender or age, while it was associated with tumor size, mitotic figures, and risk classification. The positive expression rate of Shh, Ptch, Smo and Gli-1 in GISTs with high and median risk according to NIH classification was substantially higher than those with extremely low and low risk, and the difference was significant. The positive expression rate of Shh, Ptch, Smo and Gli-1 was significantly higher in recurrent GIST than non-recurrent GIST. The 1-, 3-, and 5-year survival rates of patients with positive expression of Shh, Ptch, Smo and Gli-1 were markedly lower than those with negative expression. The positive expression of Gli-1 was an independent risk factor of patients' prognosis. Conclusion: The Shh signaling pathway was present and activated in the GIST tissues. The expression of critical proteins in the Shh signaling pathway was correlated, and they may participate in the origination and development of GIST through ligand-dependent activation. The expression of proteins in the Shh signaling pathway was associated with the reoccurrence and prognosis of GIST, particularly, the expression of Gli-1 protein may be used as an important indicator for predicting reoccurrence.

Keywords: Gastrointestinal stromal tumor (GIST), Shh signaling, targeted drug therapy

Introduction

Gastrointestinal stromal tumor (GIST) is the most common gastrointestinal mesenchymal tumors. Since GIST is not sensitive to either conventional radiotherapy or chemotherapy, radical surgery remains the major treatment, but there are still 40%-80% of patients that had relapse and metastasis in the postoperative phase [1]. Imatinib is molecular targeted drug that is currently used as a relatively successful treatment of GIST. It is a tyrosine kinase inhibitor (TKI), which significantly prolonged the overall survival of GIST patients. Imatinib has also been successfully used in the treatment of Philadelphia chromosome-positive chronic myeloid leukemia (CML) and unresectable and/or metastasized GIST in adult patients, and is considered a classical successful targeted therapy drugs The successful practice of imatinib treatment in GIST has elicited great interest of researchers to study, and to invent more and better targeted drugs in cancer therapy.

Expression of the Shh signaling pathway is associated with the development of many tumors, including digestive system cancers such as liver cancer, stomach cancer, and leukemia. The pathway consists of four parts: the ligand molecule Shh, membrane receptor Patched (Ptch), G protein-coupled phosphorylated receptor Smoothened (Smo), and the downstream nuclear transcription factor Gli. Glihas 3 subtypes, Gli-1, Gli-2 and Gli-3, and is

Table 1. Clinical and pathological features of the 129 cases of GIST patients

Clinical Characteristics	Number of patients	
	N (Total = 129)	Percent (%)
Gender		
Male	78	60.5
Female	51	39.5
Age (y)		
Tumor site		
Stomach	62	48.6
Small intestine	47	36.4
Esophagus	3	2.3
Colorectal	10	7.8
Extra gastrointestinal	7	5.4
Tumor diameter		
<2 cm	22	17.1
2-5 cm	38	29.5
6-10 cm	41	31.8
>10 cm	28	21.7.0
Mitotic counts		
≤5/50 HPF	108	83.7
6-10/50 HPF	18	14.0
>10/50 HPF	3	2.3
NIH classification		
Very low	22	17.1
Low	37	28.7
Intermediate	28	21.7
High	42	32,6
Recurrence		
No	87	67.4
Yes	42	32.6

both a transcription factor and a target gene of the Shh signaling pathway, which can increase its own activity through positive feedback [2]. Among the 3 subtypes, Gli-1 and Gli-2 are transcriptional promotor, and Gli-3 is a transcriptional repressor. The conventional manner to activate the Shh signaling pathway is through stimulation of Hh ligand that dissociates the inhibition of Ptch on Smo, which promotes Gli protein to enter the nucleus and activate downstream target genes [3]. In the absence of the corresponding ligand stimulation, the activity of Smois was inhibited by Ptch [4]. Targeting the Shh signaling pathway block the corresponding pathways in tumor may become a new way of cancer therapy [5].

Ayumi et al. [6] examined the expression of four important molecules in the Shh signaling pathway using immunohistochemistry in 31 cases of GIST tissue, and found all were expressed in GIST tissue, and their expression is different in GIST with different risk classification, but their roles remain unclear in GIST. Zhao C. et al. [7] found that, when inhibitors are used to inhibit the Shh signaling pathway, not only the spread of wild-type BCR-ABL1 CML was reduced; the growth of CML tumor cells that already generated imatinib resistance was also reduced. Of note, Imatinib is currently the first-line drug in clinical treatment of GIST.

There has been no report in the literature on the relation between Shh signaling pathway and the clinicopathological factors of GIST. In this study, we measured the expression of proteins associated with the Shh signaling pathway in GIST tissues, to analyze its relationship with clinicopathologic factors of GIST, in order to confirm the presence of Shh signaling pathway in the development process of GIST, and to further explore the relationship between important signaling pathway protein and recurrence and prognosis, to lay the foundation for a potential targeted therapy.

Materials and methods

Clinical data

The complete medical records of 129 GIST patients who went through surgical treatment at the First Affiliated Hospital of Anhui Medical University from June 2008 to December 2010 were collected. Patients received no chemotherapy or radiotherapy prior to the surgery. Age, gender, tumor location, tumor size were retrieved from the clinical history and pathology records. The study was approved by the institutional IRB and all participants gave informed consent.

Immunohistochemical methods

All specimens were fixed in 10% formalin, followed by routine paraffin-embedded sections, and HE and immunohistochemical staining. The two-step EnVision method was used, including: dewaxing the sections to water, repairing under high pressure, inactivation of endogenous peroxidase with 3% hydrogen peroxide, incubation with primary antibody overnight at 4°C, and the rest of the steps according to kit instructions, DAB coloring, and restaining with hematoxylin. All immunohisto-

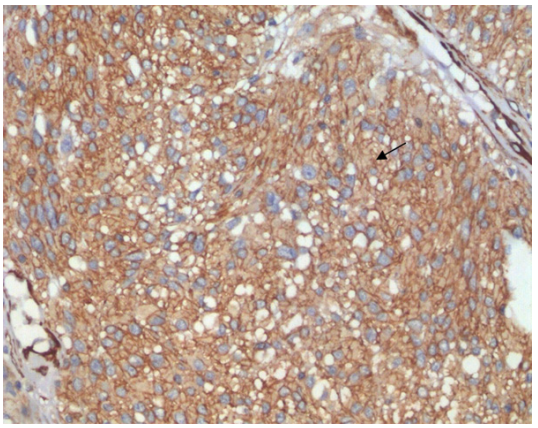

Figure 1. The immunohistochemical staining of Shh protein in the GIST tumor cell cytoplasm and on the cell membrane (200 × magnification).

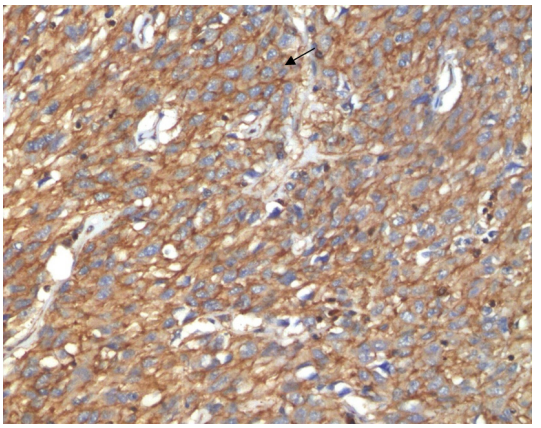

Figure 3. The immunohistochemical staining of Smo protein in the GIST tumor cell cytoplasm and on the cell membrane (200 × magnification).

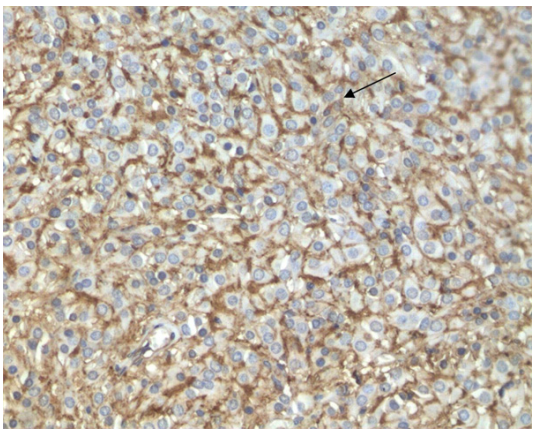

Figure 2. The immunohistochemical staining of Ptch protein in the GIST tumor cell membrane and in the cytoplasm (200 × magnification).

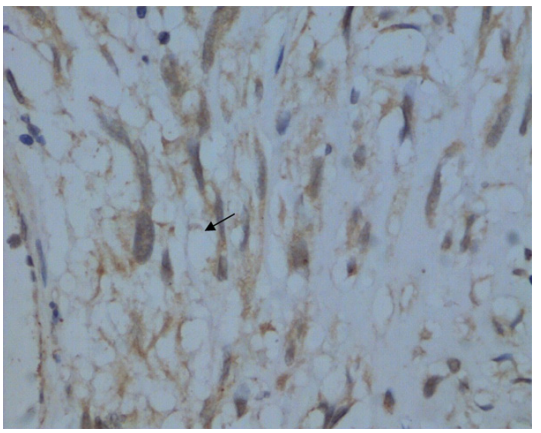

Figure 4. The immunohistochemical staining of Gli-1 protein in the GIST tumor cell cytoplasm and in the nucleus (200 × magnification).

chemical reagents including Shh antibody (sc-9024), Gli-1 antibody (sc-20687) were purchased from Santa Crus 9USA); Ptch antibody (ab39266) and Smo polyclonal antibody (ab113438) were purchased from Abcam (UK); EnVision kits were purchased from Maixin Biotech (Fuzhou, China).

Each batch of staining had known positive sections as positive control (human hepatocellular carcinoma tissue for Shh and Smo, HCC, human breast cancer tissue for Ptch, human testicular tissue for Gli-1), and PBS in place of the primary antibody as negative control.

Evaluation of immunohistochemical results

The presence of yellow or brown particles in the cell membrane and (or) cytoplasm was identified as positive for Shh, Ptch and Smo protein; the presence of yellow or brown particles in the cytoplasm and (or) nucleus was identified as positive for Gli-1 protein. Each section had 10 high power fields (× 40) selected. The evaluation criteria for positive expression are as follows: (1) Scoring according to the tumor cells staining intensity: 0 point for no colors; 1 point for pale yellow color; 2 points for yellow brown color; 3 points for dark brown color. (2) Scoring according to the percentage of positive cell counts: 0 point for positive rate of <10%; 1 point for 10%-25%; 2 points for 25%-50%; 3 points for >50%. The scores from staining intensity were multiplied by the scores from positive cell counts for each section to get the following classes: negative for results <3; positive for results >3 (3-4 as weakly positive, and 5 to 9 as strong positive).

Table 2. Correlation between expression of Shh, Ptch, Smo and Gli-1 and clinical features of GIST (n = 125)

Clinical features	Shh			Ptch			Smo			Gli-1		
	-	+	*P*	-	+	*P*	-	+	*P*	-	+	*P*
Gender			0.384			0.120			0.434			0.164
Male	26	52		20	58		31	47		37	42	
Female	15	36		17	34		21	30		29	22	
Tumor site			0.271			0.265			0.002*			0.011*
Stomach	22	40		19	43		29	33		38	24	
Small intestine	12	35		20	27		21	26		20	27	
Esophagus	1	2		1	2		1	2		1	2	
Colorectal	5	5		4	6		3	7		6	4	
Extra gastrointestinal	1	6		1	6		2	5		2	5	
Tumor diameter			0.000*			0.001*			0.001*			0.000*
<2 cm	10	12		11	11		9	13		8	14	
2-5 cm	18	20		19	19		20	18		19	19	
6-10 cm	12	29		8	33		11	30		20	21	
>10 cm	2	33		7	28		11	24		9	26	
Mitotic counts			0.000*			0.017*			0.003*			0.000*
≤5/50 HPF	17	91		29	79		37	71		42	68	
6-10/50 HPF	5	13		6	12		7	11		5	13	
>10/50 HPF	1	11		4	14		2	16		4	14	
NIH classification			0.000*			0.000*			0.000*			0.000*
Very low	9	13		8	14		5	17		7	12	
Low	12	25		16	21		16	21		10	27	
Intermediate	7	21		6	22		9	19		10	18	
High	7	35		12	30		16	26		15	27	

**P*<0.05 indicates significant association between the immunohistochemical indicator expression and clinical features tested by Chi-square linear-by-linear association.

Follow-up

The follow-up was through clinic visits, telephone calls and letters. The survival time was defined as: from the date of surgery to the follow-up ending time for surviving patients, to the time of death for deceased patients, to the last follow-up time for patients censored in follow-up. The follow-up ended on June 30, 2015. Patients died of other diseases or accidents were excluded from the study.

Statistical methods

SPSS (16.0 for window) was used for statistical analysis, chi-square test were used to analyze the relation between protein expression and clinical parameters. Pearson's was used for correlation analysis and Kaplan-Meier was used for survival analysis. Log-rank method was used to compare between groups, and Cox regression model was used for multivariate analysis. $P<0.05$ was considered statistically significant.

Results

Features of clinical and pathological data

Among the 129 cases of GIST patients, patients age ranged from 19 to 82 years (mean ± SD 58.19 ± 12.95 years). The clinical and pathological data are shown in **Table 1**.

Follow-up results

Follow-up ended on June 30, 2015. 26 patients were lost during the follow-up and 103 patients had complete follow-up data, with the rate of follow-up as 79.8%. 31 patients died due to the disease, and relapse or metastasis was confirmed in 42 cases (32.6%). The median (range) of follow-up in the surviving group was 59.0 (3.0-114.0) months.

Table 3. Relationship between expression of Shh, Ptch, Smo and Gli-1 and NIH classification of GIST (n = 125)

Clinical features	Shh			Ptch			Smo			Gli-1		
	-	+	*P*	-	+	*P*	-	+	*P*	-	+	*P*
NIH classification			0.000			0. 000			0.000			0.000
Very low and low	21	38		24	35		25	40		17	39	
Intermediate and high	14	56		18	52		19	49		25	45	

P<0.05 indicates significant association between the immunohistochemical indicator expression and NIH classification tested by Chi-square linear-by-linear association.

Table 4. Relationship between expression of Shh, Ptch, Smo and Gli-1 and recurrence of GIST (n = 125)

Clinical features	Shh			Ptch			Smo			Gli-1		
	-	+	*P*	-	+	*P*	-	+	*P*	-	+	*P*
Recurrence			0.002			0. 012			0.000			0.000
No	29	54		32	51		43	40		56	27	
Yes	4	38		7	35		6	36		6	36	

P<0.05 indicates significant association between the immunohistochemical indicator expression and recurrence tested by Chi-square linear-by-linear association.

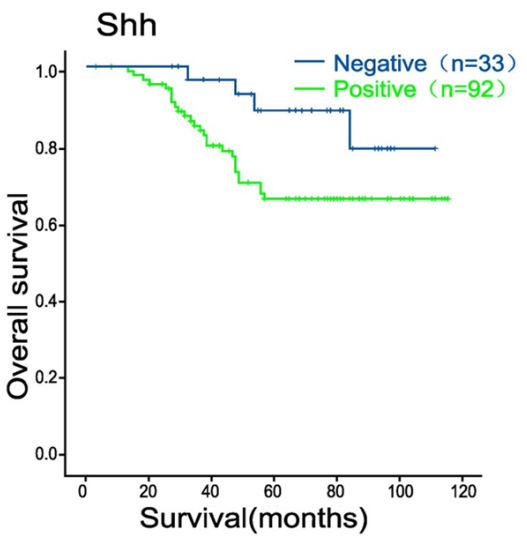

Figure 5. Overall survival of patients with GIST according to Shh expression in GIST specimens estimated by the Kaplan-Meier method. The survival rate of the patients with Shh positive tumors (n = 92) was lower than that of the patients with Shh negative tumors (n = 33); log-rank test; *P* = 0.040.

Immunohistochemical expression features

Among the 129 cases of GIST, the positive expression rates of Shh, Ptch, Smo and Gli-1 were: 72.9%, 68.2%, 64.3%, and 72.88%. Among the 87 cases of non-recurrent GIST tissues, the positive expression rates of Shh, Ptch, Smo and Gli-1 were 64.1%, 60.4%, 47.3%, and 34.5%. Among the 42 cases of recurrent GIST tissues, the positive expression rates of Shh, Ptch, Smo and Gli-1 were 91.5%, 84.3%, 86.7%, and 87.7% (**Figures 1-4**).

Correlation of Shh, Ptch, Smo and Gli-1 expression

Pearson correlation analysis showed that, in GIST tissues, Shh expression was positively correlated with Gli-1 and Smo expression (r = 0.300 and 0.001, *P* = 0.007 and 0.003, respectively); Ptch expression was positively correlated with Smo (r = 0.344, *P* = 0.000); Smo expression was positively and significantly correlated with Gli-1 expression (r = 0.416, *P* = 0.000), while Shh expression showed no correlation with Ptch expression (r = 0.145, *P* = 0.106), and Ptch expression was not correlated with Gli-1 expression (r = 0.161, *P* = 0.073).

Correlation between immunohistochemical expression and clinicopathological features

The correlation between Shh, Ptch, Smo and Gli-1 expression and clinicopathological features are shown in **Table 2**. The positive expression of Shh, Ptch, Smo and Gli-1 was associated with tumor size, mitotic count and risk classification (*P*<0.05). The larger tumor diameter, the more mitotic count, the higher NIH risk classification, and the higher positive expression rate. Smo and Gli-1 expression is also related to tumor location, histological type (P<0.05). And Shh, Ptch, Smo and Gli-1 expression has nothing to do with sex (P>0.05). Further analysis revealed that the high-risk group NIH in Shh, Ptch, Smo and Gli-1 positive

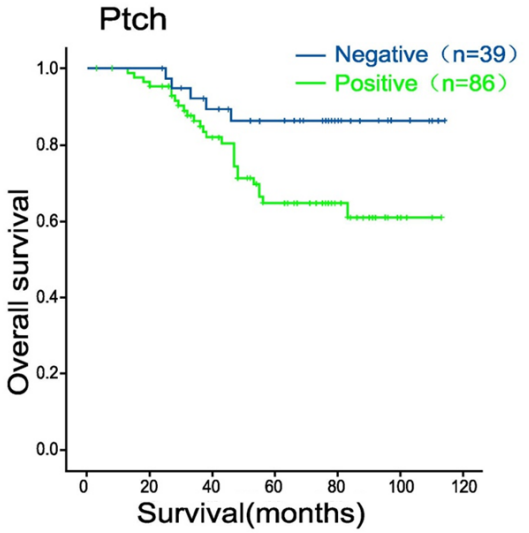

Figure 6. Overall survival of patients with GIST according to Ptch expression in GIST specimens estimated by the Kaplan-Meier method. The survival rate of the patients with Ptch positive tumors (n = 86) was lower than that of the patients with Ptch negative tumors (n = 39); log-rank test; P = 0.023.

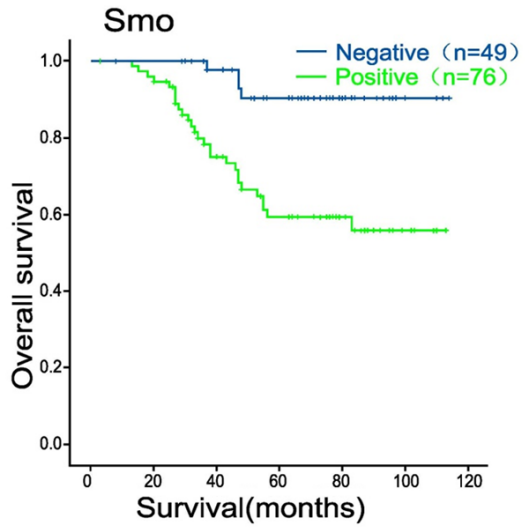

Figure 7. Overall survival of patients with GIST according to Smo expression in GIST specimens estimated by the Kaplan-Meier method. The survival rate of the patients with Smo positive tumors (n = 76) was lower than that of the patients with Smo negative tumors (n = 49); log-rank test; *P* = 0.000.

expression rate (83.9%, 80.5%, 75.9%, 67.8%) was significantly higher than the NIH is very low, positive rate of low-risk group (50.0%, 42.1%, 26.3%, 10.5%), *P* values were 0.000,0.000,0.000,0.000, the difference was statistically significant (**Table 3**).

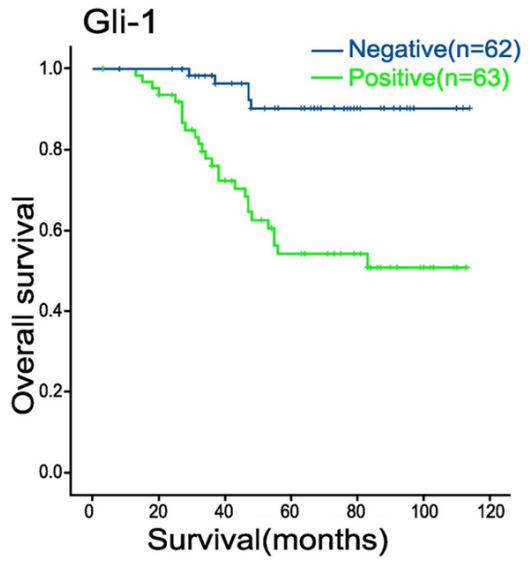

Figure 8. Overall survival of patients with GIST according to Gli-1 expression in GIST specimens estimated by the Kaplan-Meier method. The survival rate of the patients with Gli-1 positive tumors (n = 63) was lower than that of the patients with Gli-1 negative tumors (n = 62); log-rank test; *P* = 0.000.

Relationship between Shh, Ptch, Smo and Gli-1 expression and GIST recurrence

As shown in **Table 4**, During the follow-up 42 patients had definite recurrence (33.6%). Shh, Ptch, Smo and Gli-1 positive rate (90.5%, 83.3%, 85.7%, 85.7%) in relapsed GIST was significantly higher than the positive rate without recurrence of GIST (65.1%, 61.4%, 48.2%, 32.5%), *P* values were 0.002, 0.012, 0.000, 0.000, and the difference was significant.

Relationship between Shh, Ptch, Smo and Gli-1 expression and the prognosis of GIST

Univariate analysis Kaplan-Meier method prompts that Shh Ptch, Smo and Gli-1 positive group and negative group was statistically significant in survival rare. The positive expression group 1, 3, 5-year survival rate was significantly lower than the negative expression. Survival curves are shown in **Figures 5-8**.

Effect of GIST prognosis multivariate analysis

Multivariate analysis showed that the expression of Gli-1 is an independent prognostic factor, while gender, age, expression, Shh, Ptch, Smo, the mitotic, tumor location and size are not independent prognostic factors (**Table 5**).

Table 5. Multivariate analysis of survival rate of GIST (n = 125)

	B	SE	Wald	df	Sig.	Exp (B)
shh	.632	.535	1.325	1	.248	1.877
Ptch	.455	.517	.782	1	.373	1.570
Gli-1	1.362	.507	7.413	1	.007*	3.943
Smo	1.031	.571	3.248	1	.072	2.796
Gender	-.213	.379	.315	1	.575	.808
Age	.007	.014	.279	1	.598	1.008
Mitotic counts	1.031	.468	2.458	1	.287	2.583
Tumor site	1.212	.359	.326	1	.435	.789
Tumor diameter	.890	.717	.329	1	.578	1.121

*$P<0.05$.

Discussion

This study found in 129 cases of GIST that Shh signaling pathway is an important one. Hh, Ptch, Smo and Gli-1-positive rates were: 72.9%, 68.2%, 64.3%, and 72.88%. These important signaling proteins have a higher rate of positive expression, indicating that Shh signaling pathway is present in GIST tissue. Shh signal transduction pathway proteins are present in different parts of GIST tissues with diameter from small to large, from low to high mitotic, indicating that they may be involved in the occurrence and development of GIST. Ayumi et al. [6] detected by immunohistochemistry the expression of Shh pathway in 31 cases of GIST, 13 leiomyomas, 6 cases of schwannoma tissue, and found Hh, Ptch, Smo, Gli1 in the above tumor tissue. The expression results obtained were similar to our findings.

There are three common patterns of the Shh signaling pathway activation and abnormal mode of action in the process of tumor formation: [8] (1) autocrine mode, that Shh protein is produced by tumor cells or tumor stem cells; (2) paracrine mode that tumor cells secrete Shh ligand to have effect on the surrounding stromal cells, to stimulate cell growth factor secretion; (3) aberrant activation mode: abnormal activation of an important protein in Shh signaling pathways is likely to lead to abnormal activation of signaling pathways. i.e. one of the genes encoding Ptch, Smo, Gli becomes inactivated or activated, it is likely to lead to abnormal activation of signaling pathways. In our experiments, Shh protein positive staining located on the GIST cell membrane, partially located in the cytoplasm of tumor cells, Ptch protein located on the cell membrane and (or) cytoplasm; Smo protein located on the cell membrane and (or) cytoplasm. Gli-1 protein located in the cytoplasm and (or) the nucleus. Further analysis found that, in GIST tissue Shh expression of Gli-1 protein and Smo were positively correlated ($r = 0.300$ and $r = 0.001$, $P = 0.007$ and $P = 0.003$); Ptch expression of protein and Smo were positively correlated ($r = 0.344$, $P = 0.000$); protein Smo and Gli-1 expression was positively correlated, ($r = 0.416$, $P = 0.000$). The significant correlation indicates the Shh signaling pathway activation mode in GIST is ligand-dependent, and tumor cells activate Shh signaling pathways through autocrine. Shh ligands act on their own or adjacent tumor cells. We also found that Shh protein and Ptch showed no correlation ($r = 0.145$, $P = 0.106$), and Ptch protein and Gli-1 protein expression was not correlated ($r = 0.161$, $P = 0.073$). We speculated that in GIST the Shh signaling pathway has other activation mode in addition to the ligand-dependent activation. Currently there has not been report in the literature on Shh signaling pathway in GIST.

The unpredictable nature of GIST tumors is prominent, and the accurate determination of its biological behavior is still one of the major challenges. Currently the modified NIH grading criteria are used to determine the biological behavior and prognosis of GIST. A number of studies have shown that detection of Shh signaling pathway protein can be used to predict the biological behavior of tumors. As in gastric cancer, overexpression of Shh signaling pathway proteins with is closely related to age, tumor differentiation, staging, depth of invasion, lymph node metastasis and is an independent risk factor for stomach cancer prognosis [9]. In this group of GIST profile, the expression of Shh signaling pathway proteins Shh, Ptch, Smo and Gli-1 are associated with prognosis of GIST ($P<0.05$). Patients showed positive expression had shorter survival than those with negative expressions, indicating Shh expression levels can be used to predict the prognosis of GIST patients. Further multivariate analysis study also found that the expression of Gli proteins is an independent risk factor for GIST prognosis, which provides a potentially new indicator for predicting the prognosis of patients with GIST. Different from

most other tumors, using histological features is difficult to determine the biological behavior of GIST. Currently all GISTs are considered to have the potential of transforming into a malignant class of tumors [10], and the tumor size can range from small lesions to huge volume [11]. In this study we found 42 patients with definitive relapses (33.6%), the expressions of Shh signaling pathway proteins Shh, Ptch, Smo and Gli-1 are associated with recurrence ($P<0.05$). This finding is important especially for small GIST biological behavior and clinical treatment. We also take into account the sample size is small, thus further research in large multicenter is needed.

In summary, Shh signaling pathway activation is present in GIST. The expression of a variety of Shh signaling pathway proteins are correlated in GIST, and it may be involved in tumorigenesis and development through ligand-dependent activation. The positive rate of Shh signaling pathway protein expression is correlated with tumor diameter, mitotic and NIH grading, indicating that it is closely related to the development of tumor. The present study is the first to discover and explore the Shh signaling pathway protein expression and GIST prognosis and recurrence, and it may serve as an important indicator for relapse. The specific mechanism and role of Shh signaling pathway in GIST has not been reported in the literature. GIST is the most common gastrointestinal mesenchymal tumor, and its occurrence, development and metastasis pathways are different from the digestive tract malignant tumors of epithelial origin. Its transfer is common found through local invasion and metastasis in blood. The local invasion and blood metastasis of GIST is closely related to vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9), and the PI3K pathway is related to GIST too [12]. VEGF and phosphoinositide 3-kinase (PI3K) signaling pathways are important targets of therapy in other tumors where the Shh pathway plays a role. Whether Shh signaling pathway promotes tumor progression in GIST through a similar mechanism to pending further study.

Disclosure of conflict of interest

None.

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References

[1] DeMatteo RP, Lewis JJ, Leung D, Mudan SS, Woodruff JM, Brennan MF. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. Ann Surg 2000; 231: 51-58.

[2] Yang L, Xie G, Fan Q, Xie J. Activation of the hedgehog-signaling pathway in human cancer and the clinical implications. Oncogene 2010; 29: 469-481.

[3] Hui CC, Angers S. Gli proteins in development and disease. Annu Rev Cell Dev Biol 2011; 27: 513-537.

[4] Archer TC, Weeraratne SD, Pomeroy SL. Hedgehog-GLI pathway in medulloblastoma. J Clin Oncol 2012; 30: 2154-2156.

[5] Scales SJ, de Sauvage FJ. Mechanisms of Hedgehog pathway activation in cancer and implications for therapy. Trends Pharmacol Sci 2009; 30: 303-312.

[6] Yoshizaki A, Nakayama T, Naito S, Wen CY, Sekine I. Expressions of sonic hedgehog, patched, smoothened and Gli-1 in human intestinal stromal tumors and their correlation with prognosis. World J Gastroenterol 2006; 12: 5687-5691.

[7] Zhao C, Chen A, Jamieson CH, Fereshteh M, Abrahamsson A, Blum J, Kwon HY, Kim J, Chute JP, Rizzieri D, Munchhof M, VanArsdale T, Beachy PA, Reya T. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature 2009; 458: 776-9.

[8] Patil MA, Zhang J, Ho C, Cheung ST, Fan ST, Chen X. Hedgehog signaling in human hepatocellular carcinoma. Cancer Biol Ther 2006; 5: 111-117.

[9] Niu Y, Li F, Tang B, Shi Y, Hao Y, Yu P. Clinicacopathological correlation and prognostic significance of sonic hedgehog protein overexpression in human gastric cancer. Int J Clin Exp Pathol 2014; 7: 5144-5153.

[10] Chandrasekhara V, Ginsberg GG. Endoscopic management of gastrointestinal stromal tumors. Curr Gastroenterol Rep 2011; 13: 532-539.

[11] Joensuu H, Hohenberger P, Corless CL. Gastrointestinal stromal tumour. Lancet 2013; 382: 973-983.

[12] Qi YJ, Meng XL, Wang ZG. The expression of Ets-1, MMP-9, VEGF in GIST tissues and its significance. Shandong Medicine 2010; 50: 50-51.