RESEARCH COMMUNICATION

Expression of DOG1, CD117 and PDGFRA in Gastrointestinal Stromal Tumors and Correlations with Clinicopathology

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Abstract

Objective: To discuss the significance of DOG1, CD117 and PDGFRA in the diagnosis of gastrointestinal stromal tumors (GISTs), and analyze their correlations with clinicopathological features and risk ranking. Method: DOG1, CD117 and PDGFRA were detected with IHC Envision ldpe-g-nvp in 63 GISTs and 43 cases of non-GISTs, and analyzed for relations with clinicopathological factors (gender, age, location, tumor size, mitotic phase, histology) and risk degree. Results: The positive expression rate of DOG1, CD117 and PDGFRA in GISTs was 84.1% (53/63), 90.5% (57/63), 53.2% (33/63), respectively. Among the 6 CD117 negative cases, all were DOG1 positive and 5 were PDGFRA positive. Rates in patients with non-GISTs was 11.6%, 16.3%, 6.98%, respectively. Expression of DOG1 and PDGFRA demonstrated no significant variation with gender, age, position, tumor size, mitotic phase, histology, and risk rank. However, CD117 was related with position and histology (P=0.008 and P=0.045), those in the mesentery having a higher positive rate than those derived from stomach, small intestine, colon and rectum (50.0% vs 94.7%, P=0.008). Furthermore CD117 was also highly expressed in spindle and epithele types. Conclusions: DOG1 had a good sensitivity and specificity as a kind of newly discovered marker, especially for KIT negative GISTs. However, DOG1, CD117 and PDGFRA cannot be used for assessing the rish of patients.

Keywords: Gastrointestinal stromal tumor - KIT - PDGFR alpha - clinicopathological features - risk ranking

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Introduction

Gastrointestinal stromal tumors (GISTs) are a special kind of tumors which is derived from mesenchymal tissues of gastrointestinal tract. GIST is the most common mesenchymal tumors of the digestive tract. GISTs are often CD117 positive, and are c-kit or platelet derivative growth factor receptor (PDGFRA) gain-of-function mutant frequently. In recent years, with the development of effective targeted therapies for GISTs, the prognosis of GISTs patients is significantly improved and accurate diagnosis of GISTs become important, especially for the KIT absent GISTs. DOG1 (discovered on GIST-1) is a newly identified marker of GISTs, which is almost as sensitive as KIT (Miettinen et al., 2009). DOG1 is a kind of chloride ion channel proteins regulated by calcium, however, the function of DOG1 remains unclear.

The high expression of DOG1 in GISTs indicates its importance in the tumorigenesis and tumor developments, and DOG1 may be a potential marker for tumor diagnosis. The high sensitivity and specificity makes DOG1 an important diagnose evidence, the combined detection of GISTs, CD117, and PDGFRA can provide more accurate diagnose evidences both in pathology and in clinic. The probability that DOG1 may be a potential target for GISTs therapy makes it possible to develop new targeted drugs and to identify the patients who can benefit from this therapy. However, there are not enough and thorough studies of DOG1, the biological functions, the mechanism of its high expression in GISTs, and the correlation of DOG1 expression with GIST1 tumorigenesis have not been confirmed. Further research should be performed to achieve a breakthrough.

There is a bottleneck in the study of GISTs, and an urgent matter of the moment. The discovery of DOG1 may provide new methods for diagnosis and treatment in various hospitals. There are only a few researchers have detected the expression of DOG1 in tissues by immunohistochemistry. In the research, we detected the expression of DOG1 in 63 tissues of patients with GISTs and 43 tissues of patients with non-GISTs by immunohistochemistry to explore the clinical significance of the immunohistochemistry results and relative clinic pathologic factors and the correlation with the tumor stages. We suppose to improve the detection rate of GISTs and reduce the misdiagnose rate.

Materials and Methods

Materials

63 tissues of patients with GISTs were collected in the Third Affiliated Hospital of Harbin Medical University

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from January 2002 to December 2008. All these samples were medical records complete and well-diagnosed; all these samples were primary tumors, among them, male 31 cases, female 32 cases, the rate of males and females was 1:1, between the age of 24-83 (median age 56), tumor size were between 1.5-29 cm(median size 9.0 cm), lesions locations: stomach 34 (54%), small intestine 13 (21%), rectum 7 (11%), colon 3 (5%), and other positions (mesenterium, enterocoelia, and omentum) 6 (10%). Of histologic types, spindle type 52 (83%), epithelial type 6 (10%), mixed type 5 (8%). According to the NIH risk table of GIST (2008) (Table 1), these samples were classified into four stages: very low risk 4 (6%), low risk 13 (1%), intermediate risk 19 (30%), high risk 27 (43%). Besides, we collected 43 tissues of well-diagnosed non-GISTs mesenchymal tumors, among them, there were 25 schwannomas, and 6 leiomyosarcomas. Monoclonal antibodies of DOG1, CD117 and PDGFRA and IHC kits were brought from Zhongshanjinqiao Co. Ltd.

methods

All the samples were fixed in 10% neutral formalin and desiccated and embedded in paraffin and sectioned to 3μm. The antibody dilution and process of staining were performed according to the instructions. PBS was used as a negative control to primary antibodies. The results were observed under microscope. Result assessments the staining of GOD1 were mainly localized in cyroplasma, a few were localized in the cytomembrane, CD117 was mainly in the cytoplasma and a few in the cytomembrane, too, and PDGERA was also mainly in the cytoplasma. Cells were categorized according to the positive rate: negative(-), the number of positive cells < 5%, weak positive(+), pale brown particles, the number of positive cells 5%-25%, positive(++), brown particles, the number of positive cells 25%-50%, strong positive(+++), dark brown particles, the number of positive cells > 50%.

Statistical analysis

The data were analyzed using SPSS13.0, the enumeration data were compared with χ^2 test, data were considered to be statistically different when $P \leq 0.05$.

Results

Localization of DOG1, CD117, AND PDGFRA in tissues of GISTs patients (Table 2): In the samples of GISTs, the staining of GOD1 were mainly localized in cyroplasma, a few were localized in the cytomembrane, CD117 was mainly in the cytoplasma and a few in the cytomembrane, too, and PDGERA was also mainly in the cytoplasma. All these staining were distributed dispersedly (Figure 1). The positive rate of DOG1 was 84.13%, among which were 18 weak positive (+) (28.57%), 12 positive (++) (19.1%), 23 strong positive (+++) (36.5%). The positive rate of CD117 was 90.48%, higher than DOG1, and the positive rate of PDGFRA was 52.4%. When DOG1 and CD117 were compared with PDGFRA, $P < 0.01$, and data were statistically different. In the 6 CD117 negative samples, all of them were DOG1 positive, and 5 were PDGFRA positive.

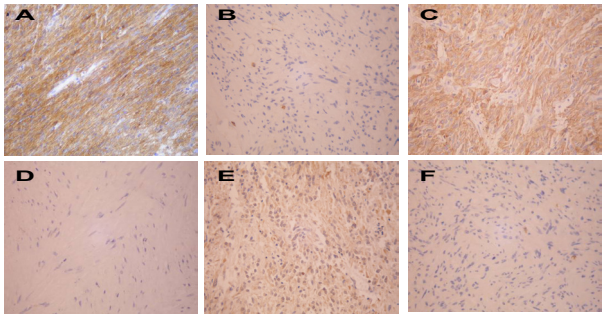

Figure 1. The Expressions of DOG1, CD117 and PDGFRA in the Gastrointestinal Stromal Tumor (GIST) Tissues. A. DOG1 was positively expressed in GIST tissue. B. DOG1 was negatively expressed in GIST tissue. C. CD117 was positively expressed in GIST tissue. D. CD117 was negatively expressed in GIST tissue. E. PDGFRA was positively expressed in GIST tissue. F. PDGFRA was negatively expressed in GIST tissue (×400)

Table 1. The Risk Grading of the Patients after Primary GIST Ectomy

Caryocinesia (n/50 highpower fields)	risk grading
≤5	extreme low
≤5	low
>5	moderate
12-6-10	moderate
≤5	moderate
any number	high
any number	high
>10	high
>5	high
>5	high
≤5	high

Table 2. The Expressions of DOG1, CD117 and PDGFRA in GISTs Tissues

Negative cases	Positive cases	Positive rate	x^2 value	P value
10	53	84.13%	1.145	0.285
6	57	90.48%	22.4	0.000[b]
30	33	52.38%	14.651	0.000[d]

DOG1, CD117, and PDGFRA in non-GIST patients (Table 3): in samples of 3 schwannoma and 1 leiomyoma the expression rate of DOG1 was 11.63% and 16.28%, respectively, lower than CD117. In 1 DOG1 positive sample of leiomyoma, the expression rate of PDFGRA was 6.98%.

Correlations of pathological factors and risk grades (Table 4): the results showed the expressions of DOG1 and PDGFRA were not statistically different and had no correlations with the sex, age, position, tumor size, mitosis, histologic type, and risk grade, however, the expression of CD117 was related with the position and histologic type: in the mesenterium, enterocoelia, and omentum, the expression was lower than stomach, small intestine, and colon, and CD117 was highly expressed in spindle and epithelial cells. Data was statistically significant.

Discussion

GISTs are a common kind of tumor derived from

Table 3. The Expressions of DOG1, CD117 and PDGFRA in non-GISTs Tissues

case	DOG1		CD117		PDGFRA	
	-	+	-	+	-	+
25	22(88.00%)	3(12.00%)	21(84.00%)	4(16.00%)	23(92.00%)	2(8.00%)
12	11(91.67%)	1(8.33%)	10(83.33%)	2(16.67%)	11(91.67%)	1(8.33%)
6	5(83.33%)	1(16.67%)	5(83.33%)	1(1.67%)	6(100%)	0(0%)
43	8(88.37%)	5(11.63%)	36(83.71%)	7(16.28%)	40(93.02%)	3(6.98%)

Table 4. The Relationship Between the Expressions of DOG1, CE117 and PDGFRA in GISTs and Clinincal Pathological Factors, and Risk

Clinical pathological characteristc	DOG1	x^2	P	CD117	x^2	P	PDGFRA	x^2	P
gender									
male(n=31)	26(83.87%)	0.003	0.956	28(90.32%)	0.002	0.967	16(51.61%)	0.104	0.904
female(n=32)	27(84.38%)			29(90.63%)			17(53.13%)		
age									
≤50 (n=20)	16(80.00%)	0.374	0.541	19(95.00%)	0.696	0.404	12(60.00%)	0.682	0.409
>50 (n=43)	37(86.05%)			38(88.37%)			21(48.84%)		
location									
stomach (n=34)	27(79.41%)	4.433	0.351	32(94.12%)	13.8	0.008	19(55.88%)	0.883	0.927
small intestine(n=13)	13(100%)			13(100%)			6(46.15%)		
rectum(n=7)	5(71.43%)			6(85.71%)			3(42.86%)		
colon(n=3)	3(100%)			3(100%)			2(66.67%)		
others(n=6)	5(83.33%)			3(50.00%)			3(50.00%)		
Tumor size (cm)									
≤2(n=4)	4(100%)	6.182	0.103	3(75.00%)	3.382	0.336	3(75.00%)	1.837	0.607
2.1-5(n=18)	12(66.67%)			18(100%)			10(55.56%)		
5.1-10(n=26)	23(88.46%)			23(88.46%)			14(53.85%)		
>10(n=15)	14(93.33%)			13(86.67%)			6(40.00%)		
Caryocinesia (n/50 HPF)									
≤5(n=43)	35(81.40%)	1.021	0.6	40(93.02%)	2.397	0.302	24(55.81%)	1.304	0.521
6-10(n=17)	15(88.24%)			15(88.24%)			7(41.18%)		
>10(n=3)	3(100%)			2(85.00%)			2(45.00%)		
Histology type									
Spindle type	43(82.69%)	1.026	0.599	48(92.31%)	6.223	0.045	25(48.08%)	2.406	0.3
Epithelium type	5(83.33%)			6(100%)			4(66.67%)		
mixed type	5(100%)			3(60.00%)			4(80.00%)		
risk									
extreme low	4(100%)	7.764	0.051	3(75.00%)	3.758	0.289	3(75.00%)	1.151	0.765
low	10(76.92%)			13(100%)			7(53.85%)		
moderate	13(68.42%)			18(94.74%)			9(47.37%)		
high	26(96.30%)			23(85.19%)			13(48.15%)		

gastrointestinal mesenchyma, which is presented as spindle and epithelial cells, and sometimes pleomorphic cells arranged in cords or diffuse histologically. GISTs express the gene product KIT (CD117) of c-Kit, and are a mutant c-Kit or PDGFRA induced tumor. According to foreign reports, the incidence of GISTs is 1-2/100, 000, under 3% of total gastrointestinal tumors, however, GISTs is the most common mesenchymal tumors of the digestive tract. It can appeared anywhere of the digestive tract, most commonly in stomach (60%-70%), small intestine (20%-30%), or rectum and colon(5%), esophagus fewer than 5%, and sometimes in omentum and mesenterium (Miettinen et al., 2005; Dow et al., 2006; Stamatakos et al., 2009). The age peak of incidence is 55-65, male slightly higher than female or almost equal for both sexes (Bórquez Ma et al., 2008; Corless et al., 2008). Our data showed that lesions were located mainly in stomach, 54% (34 samples), small intestine, 21% (13 samples), rectum, 11% (7 samples), colon, 5% (3 samples), and other positions (omentum, enterocoelia, and mesenterium), 10% (6 samples), male, 31 samples, female, 32 samples, sex ratio 1:1, age between 24-83, median age 56, similar to former literatures.

As a specific marker of GISTs, CD117 has good sensitivity, and was highly expressed in most GISTs in a diffused pattern. The positive rate is 85%-94%, that is to say, nearly all the GISTs express c-Kit, so CD117 is used as a marker of GISTs. In our studies, the positive rate of CD117 was 90.48%, in agreement with references. CD117 can be a target for therapy, and the use of TKIs for the inhibition of KIT and PDGFRA kinase has revolutionized the treatment of GISTs, so this specific diagnose is a potential therapy of patients. But CD117 did not show the absolute specificity and about 5% GISTs are CD117 absent. What's more, some other tumors like seminoma, mastocytoma, and malignant melanoma also express CD117 (Hornick and Fletcher, 2002), that made the diagnose of GISTs difficult.

Heinrich reported the gain-of-function mutation of PDGFRA in GISTs in 2003 (Heinrich et al., 2003), and further confirmed the expression of PDGFRA protein by western blot, making PDGFRA a new marker of GISTs diagnose. Our results showed that the positive rate of

PDGFRA was 52.38%, and PDGFRA showed a low sensitivity. Compared with CD117, there was statistically different between them, and $P < 0.01$. Among the 6 CD117 negative samples, 5 samples were PDGFRA positive, showed that PDGFRA was highly expressed in CD117 negative GISTs, in agreement with references. It can be indicated that the combined detection of CD117 and PDGFRA will have clinical significance, but PDGFRA is not the specific marker of GISTs, there are no reliable antibodies of PDGFRA for paraffin embedded tissues, so the reports of PDGFRA immunohistochemical staining were rare and the results are disaccord (Medeiros et al., 2004; Pauls K et al., 2005; Rossi et al., 2005; Miettinen and Lasota, 2006; Peterson et al., 2006). The diagnose value of PDGFRA is minimized for also the reason that other tumors (leiomyosarcoma and synovial sarcoma) may be PDGFRA positive.

Because the first generation and second generation tyrosine kinase inhibitor drug are effective, the accurate diagnosis is very important. But the diagnose of GISTs is still limited in the immunohistochemistry detection of CD117 and CD34 and the combined detection of anatomical localization and histomorphometric analysis. In about 5% histomorphology uncertain GISTs, the diagnose is difficult for the negative expression of CD117. Though the detection of KIT and PDGFRA mutations are helpful, this process rises the cost and time of diagnose, and the detection of c-Kit is not reliable in most domestic hospitals. These limitations drive researchers to find other makers for GISTs.

DOG1 is a newly-discovered marker of GISTs, which is highly expressed in GISTs, was screened by West (West et al., 2004). DOG1 is located in the 11q13 region, contain 26 exons, and encodes an 114kDa protein including 960 amino acids (Caputo et al., 2008; Yang et al., 2008). The protein has 8 transmembrane function domains, and is supposed to be a calcium regulated chloride ion channel protein (Carles et al., 2006). Mutant DOG1 has not been found (Miwa et al, 2008). However, the function of DOG1 is not clear.

In recent years, the study of DOG1 is becoming a hotspot of GISTs research. Many foreign reports showed that the sensitivity of DOG1 and KIT are almost the same (≥95%), and the two factors have consistency. When detected together with CD117, it has strong complementarity in the diagnose of GISTs. And domestic studies reported that the positive expression rate of DOG1 was 96.1%, in agreement with foreign reports. Our study showed that the positive rate of DOG1 in GISTs was 84%, which was as reported by Espinosa (Espinosa et al., 2008) and Yin Mujun. Though the positive rate was lower than CD117, but DOG1 was positive expressed in the 6 CD117 negative samples, indicating that DOG1 could be used as a supplement means for GISTs diagnose, and as a potential evidence for some cases. Strong positive samples had a higher sensitivity (36.5%) than weak positive samples (28.6%) and positive samples (19%). There was statistical significance between the expression rates of DOG1 and PDGFRA, and DOG1 had a higher sensitivity than PDGFRA. DOG1 can be used as a better marker of GISTs, for it was detected poorly expressed in non-GISTs (schwannoma 12%, leiomyoma 8%, and leiomyosarcoma 17%).

There are only a few literatures focusing on the relationship of clinic pathologic parameters, risks and DOG1, CD117, and PDGFRA, even more difficult, there are big gaps in these results. Zhangzhuxue reported that the expressions of PDGFRA and CD117 were significantly different between histomorphological types: PDGFRA had a high expression rate in mixed cellularity (81%), while CD117 was highly expressed in spindle cells (98%). Kang et al. (2010) reported that the expressions of DOG1 and PDGFRA were related with tissue types, both of them were highly expressed in spindle cells, and CD117 was highly expressed in female patients. While PDFGRA was related with position, the expression rate in non-small intestine increased significantly. Espinosa and Zhen Liying (Espinosa et al., 2008) reported that there were no relation between the biological factors (type of mutation, mutation site, tumor size, and tumor stage) and expression of DOG1. Yin Mujun reported that the expression of DOG1 was related with the tumor position, the enrichment of cells, nuclear atypia and the Fletcher risk grade. Our studies showed that the expression of DOG1 was not significantly different in various sexes, ages, positions, tumor sizes, nuclear atypia, and histomorphological types, which was in agreement with Espinosa and Zhen Liying (Espinosa et al., 2008). There was no relation with the risk grades, however, the P value was 0.051, so it worth further research. The expression of CD117 showed significant differences between various positions and histomorphological types, the expression in stomach, small intestine, and colorectal were higher than other parts (omentum, enterocoelia, and mesenterium), and was highly expressed in spindle cells and epithelial cells, while the expression of PDGFRA was not related with clinic pathologic parameters and risk grades, there are differences between our results and other groups, further study is needed.

Great progress has been made in the clinical features; diagnosis and treatment, but there are lots questions need to be dissolved, especially in the mechanism of tumorigenesis, biology behavior prediction and more effective treatments. Thorough studies on CD117 and PDFGRA in GISTs are thorough, but the relationship between the expression and clinical pathological factors and risk grade are not clear. The biological function of DOG1 and the mechanisms of the highly expression in GISTs are still not clear, and further studies are needed. But DOG1 can be used as a specific marker of GISTs is beyond doubt, and for CD117-negative patients, the detection of DOG1 has a diagnostic value. We believe that as the research of GISTs go further, the roles of DOG1, PDFGRA, and CD117 in the mechanisms, diagnose, treatments, and prognosis will become clear. This will be important for the clear diagnosis, better targeted drugs, extending the life span, and improving the prognosis of patients.

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