Expression of Cyclin D1, CDC25B, and p27 protein in gastric carcinoma and its clinical significance

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ABSTRACT

Background: Gastric cancer is a common digestive tract tumor in our China, with high morbidity and mortality. Similar with other tumors, the occurrence of gastric cancer was also a complex pathophysiological process, regulated by a variety of oncogenes and tumor suppressor gene. **Aims and Objectives:** This study aims to investigate the expression of cyclin D1, CDC25B, and p27 in gastric carcinoma and to study their relationship with the occurrence and development of gastric cancer. **Materials and Methods:** SP immunohistochemical and Western blot analysis were used to detect the expression of cyclin D1, CDC25B, and p27 in 42 cases of gastric carcinoma, 42 cases of paracancer, and 42 cases of normal gastric tissue and then their relationship with clinical and pathological factors was analyzed. **Results:** (1) The expression of cyclin D1 increased gradually in normal gastric tissue, paracancer, and gastric carcinoma ($P > 0.05$). The expression of CDC25B was higher in gastric carcinoma ($P < 0.05$) while p27 was lower ($P < 0.05$). (2) The result of Western blot shows that the expression of cyclin D1 and CDC25B was higher in gastric carcinoma than that in normal gastric tissue while p27 lower in gastric carcinoma ($P < 0.05$), same with immunohistochemical. (3) The expressions of cyclin D1 and CDC25B had correlation with lymph nodes metastasis, and p27 had correlation with degrees of pathological differentiation, invasion depth, and lymph nodes metastasis. (4) There was a positive relationship between the expression of cyclin D1 and CDC25B in same sample ($r = -0.392$, $P < 0.05$). **Conclusion:** Expression of cyclin D1, CDC25B, and p27 protein can be helpful in the prediction of the biological behavior and prognosis of gastric carcinoma.

Key words: CDC25B, Cyclin D1, Gastric carcinoma, p27, SP immunohistochemical, Western blot

INTRODUCTION

Gastric cancer is a common digestive tract tumor in our China, with high morbidity and mortality. Similar with other tumors, the occurrence of gastric cancer was also a complex pathophysiological process, regulated by a variety of oncogenes and tumor suppressor gene. The occurrence of tumor is associated with abnormal of cell cycle regulation and has been confirmed in many kinds of tumor tissue. Cyclin D1 is one of the important cell cycle proteins, the overexpression of which can cause cell proliferation and occurrence of tumor. CDC25B and p27 are important cell cycle regulatory factors, studied in many tumors such as lung cancer and colorectal cancer tissues. SP immunohistochemical and Western blot analysis were used to detect the expression of cyclin D1, CDC25B, and p27 in 42 cases of gastric carcinoma, 42 cases of paracancer, and 42 cases of normal gastric tissue and then their relationship with clinical and pathological factors was analyzed, so as to provide a new reference index for the evaluation of gastric cancer biological behavior and prognosis evaluation.

Aims and objectives

This study aims to investigate the expression of cyclin D1, CDC25B, and p27 in gastric carcinoma and to study

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their relationship with the occurrence and development of gastric cancer.

MATERIALS AND METHODS

Specimens collection

All specimens were collected from Chengde Medical College in 2008–2014. All of the primary patients, without chemotherapy, radiotherapy, or hormone therapy before surgical resection including 42 cases of patients in this study, 31 cases of male and 11 cases of female, age 34~84, and the average age 59.35 years old, high differentiation 15 cases, middle differentiation 13 cases, and poor differentiation 14 cases. Select about 1 cm from edge of tumor tissue as paracancer, >5 cm away from the tumor edge as normal mucosa tissues. Collect the above fresh gastric carcinoma tissue and normal gastric tissue, liquid nitrogen preservation, used in Western blot experiments.

Reagent

Rabbit anti-human cyclin D1, CDC25B, and p27 monoclonal antibody were bought from Chinese Fir Jinqiao Biological Technology Co., SP kit and DAB chromogenic agent were bought from Fujian New Technology Co., Ltd.

Immunohistochemistry

SP method was according to the kit instructions, positive control groups were provided by Reagent Company and PBS was regarded as negative control groups. The positive staining for cyclin D1, CDC25B, and p27cells was expressed as dark brown granules, which were mainly located in cell nucleus under microscopy. The percentage of positive cells was divided into five grades (percentage cores): ≤5%=score 0; 6–20%=score 1; 21–50%=score 2; 51–75%=score 3; and >75%=score 4, the scores ≤1 were defined as negative and >1 as positive. All slides were read by two experienced pathologists.

Western blot

Fresh gastric tissue, cut up, add PMSF, extraction of total protein, SDS polyacrylamide gel electrophoresis, after TBST liquid containing 5% skimmed milk closed. Each membrane, respectively, incubated with anti-1. PVDF membrane was washed 3 times, 15, 10, and 10 min, dilute second antibody at 1:5000, add second antibody and keep 1 h, developing and fixing. Read the stripes width and gray value by ImageJ image. The data were analyzed by statistical Microsoft.

Statistical analysis

SPSS18.0 statistical package was used to analyze data. The relationship between protein expression and clinical pathological indicators is determined using Chi-square test, $P<0.05$ as statistically significant. Western blot results were expressed with $\overline{x} \pm S$, with independent sample t-test (independent sample t-test), according, $P<0.5$, to outstanding difference.

RESULTS

The expression of cyclin D1, CDC25B, and p27 protein in gastric carcinoma, paracancer, and normal gastric tissue

The expression rate of cyclin D1 in normal gastric tissue, paracancer, and gastric carcinoma was 21.43%, 23.81%, and 38.11%, having no significant difference ($\chi^2=3.402$, $P=0.182$), but we can see the increase trend. The expression rate of CDC25B in normal gastric tissue, paracancer, and gastric carcinoma was 0.00%, 14.29%, and 50.00%, with significant difference ($\chi^2=33.091$, $P=0.000$). The expression rate of p27 in three groups was 61.90%, 30.95%, and 28.57%, with significant difference ($\chi^2=12.056$, $P=0.002$) (Table 1 and Figure 1).

The relationship between cyclin D1, CDC25B, p27, and clinical pathological indicators

The relationship between cyclin D1, CDC25B, p27, and clinical pathological indicators is shown in Table 2. The expressions of cyclin D1 and CDC25B had correlation with lymph nodes metastasis, and p27 had correlation with degrees of pathological differentiation, invasion depth, and lymph nodes metastasis ($P<0.05$).

Table 1: The expression of cyclin D1, CDC25B, and p27 protein in gastric carcinoma, paracancer, and normal gastric tissue

	Cases	Cyclin D1		P	CDC25B		P	p27		P
		Negative	Positive		Negative	Positive		Negative	Positive	
Normal gastric tissue	42	33	9	0.50★	42	0	0.013★	16	26	0.04★
Paracancer	42	32	10	0.07c	36	6	0.000a	29	13	0.020
Gastric carcinoma	42	26	16	0.20i	21	21	0.000▲	30	12	0.500

★: Normal gastric tissue and paracancer, ●: Normal gastric tissue and gastric carcinoma, ▲: Paracancer and gastric carcinoma
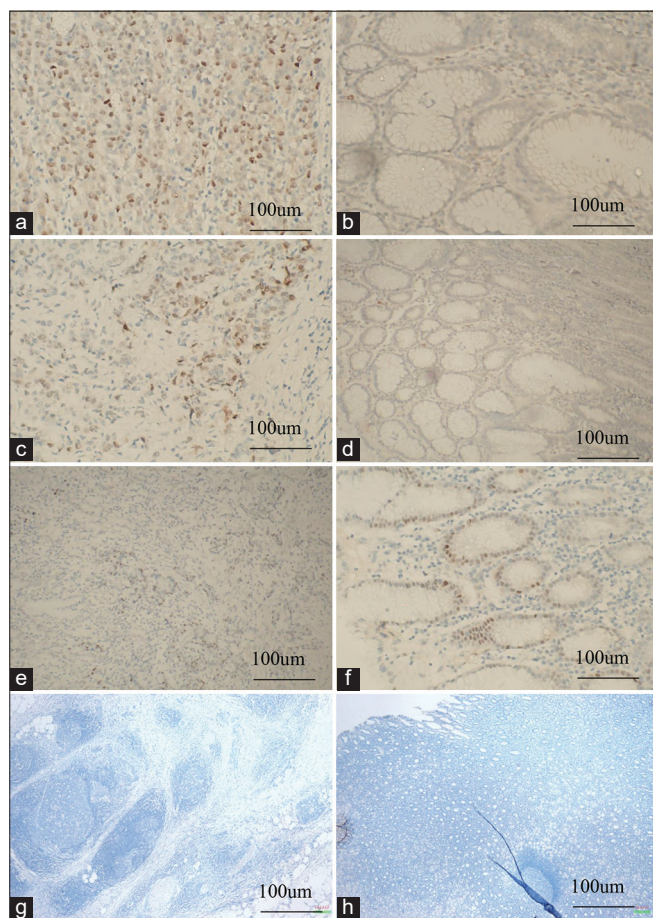
Figure 1: (a) High expression of cyclin D1 in gastric carcinoma, (b) low expression of cyclin D1 in normal gastric tissue, (c) high expression of CDC25B in gastric carcinoma, (d) low expression of CDC25B in normal gastric tissue, (e) low expression of p27 in gastric carcinoma, (f) high expression of p27 in normal gastric tissue, (g and h) negative control

The expression of cyclin D1, CDC25B, and p27 protein in gastric carcinoma by Western blot
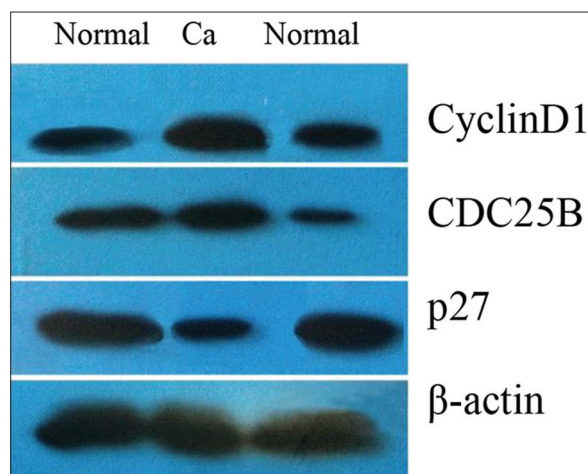
Western blot shows that the expression of cyclin D1 and CDC25B in normal gastric tissue was 0.267 ± 0.026 and 0.385 ± 0.044, respectively, significantly lower than that in gastric carcinoma (0.649 ± 0.052 and 0.945 ± 0.059) ($P<0.05$), the expression of p27 in normal gastric tissue was 0.841 ± 0.073, higher than that in gastric carcinoma (0.291 ± 0.019) ($P<0.05$) (Figure 2).

The relationship between cyclin D1, CDC25B, and p27 in gastric carcinoma

In 42 cases of gastric carcinoma, the expression of cyclin D1 is positively correlated with CDC25B ($r=0.392$, $P<0.05$), (Table 3).

DISCUSSION

As all known, cyclin D1 is a cell cycle protein, at 11 q13 chromosomes, the protein product contains 29 amino acid residues, cyclin D1 plays an important role in the

Figure 2: The expression of cyclin D1, CDC25B, and p27 protein in gastric carcinoma by Western blot

cell cycle and a major role in the transformation of the G/S period is regarded as a positive control factor. At the same time, cyclin D1 is regulated by the growth factors and other factors. Under normal physiological condition, cyclin D1 can make pRb phosphorylation by combining with CDK4and pRb, and then release E2F, promoting cell proliferation. The overexpression of cyclin D1 can lead to abnormal cell regulation and promote the occurrence of tumor.[1-3] The overexpression of cyclin D1 was found in a wide variety of tumor tissues, such as head-and-neck squamous cell carcinoma,[4,5] gastric carcinoma,[6,7] and bile duct carcinoma.[8,9] The report of cyclin D1 in gastric carcinoma is less, Arber et al.,[5] detected the expression of cyclin D1 protein in gastric cancer tissue and compared with tissue adjacent to carcinoma, the results show that the cyclin D1 expression is higher in gastric cancer tissue, in our study, the expression of cyclin D1 increased gradually in normal gastric tissue, paracancer, and gastric carcinoma. Western blot shows that the expression of cyclin D1 was higher in gastric carcinoma than that in normal gastric tissue, and the expressions of cyclin D1 had correlation with lymph nodes metastasis. So, it can be concluded that the expression of cyclin D1 tends to increase the increasing degree of malignancy.

CDC25B belongs to Sue/tyrosine enzyme, mainly plays a role to maintain normal physiological function of cells, may be associated with embryonic development. The genes located in 20p13,[10-13] recently, reports showed that the overexpression of CDC25B associated with the development and prognosis of tumor.[14] Our study showed that the expression rate of CDC25B in normal gastric tissue, paracancer, and gastric carcinoma was 0.00%, 14.29%, and 50.00%, with significant difference, Western blot also suggests of high expression of CDC25B in gastric cancer tissue. P27 belongs to the cell cycle dependent kinase inhibitors (cyclin-dependent kinase inhibitors), the

Table 2: The relationship between cyclin D1, CDC25B, p27, and clinical pathological indicators

Characteristics	n	Cyclin D1 Positive rare (%)				CDC25B 阳性率 (%)				p27 阳性率 (%)			
		Positive	Negative	Rate	P	Positive	Negative	Rate	P	Positive	Negative	Rate	P
Gender													
Male	31	13	18	41.93	0.314	16	15	51.61	0.500	7	24	22.58	0.146
Female	11	3	8	27.27		5	6	45.45		5	6	45.45	
Age													
≤59	22	5	17	22.72	0.053	10	12	45.45	0.379	7	15	31.82	0.443
>59	20	11	9	55		11	9	55		5	15	25	
Differentiate													
High	15	3	12	20	0.194	5	10	33.33	0.166	8	7	53.33	0.02
Middle	13	6	7	46.15		9	4	69.23		3	10	23.08	
Poor	14	7	7	50		7	7	50		1	13	7.14	
Depth of invasion													
None full	15	3	12	20	0.06	5	10	33.33	0.09	8	7	53.33	0.01
Full thickness	27	13	14	48.15		16	11	59.25		4	23	14.81	
Lymph node metastasis													
Positive	18	11	7	61.11	0.009	13	5	72.22	0.01	1	17	5.56	0.004
Negative	24	5	19	20.83		8	16	33.33		11	13	45.83	

Table 3: The relationship between cyclin D1, CDC25B, and p27 in gastric carcinoma

		CDC25B		p27	
		+	−	+	−
Cyclin D1	+	12	4	8	8
	−	9	17	4	22

expression of p27 was found low in many tumors.[15,16] In our study, P27 low expression had correlation with degrees of pathological differentiation, invasion depth, and lymph nodes metastasis. Suggesting the low p27 may be associated with the poor prognosis of gastric adenocarcinoma.

The correlation between cyclin D1 and p27 has been researched in few studies, as mentioned above, cyclin D1 can make pRb phosphorylation by combining with CDK4 and pRb, and then release E2F, promoting cell proliferation. As a tumor suppressor gene, p27 can inhibit the formation of the complex. CDC25B is a tumor source protein, can remove tyrosine residues in CDK1, promoting the transformation of G2-M.[17-20] Our experiment results show that in 42 cases of gastric carcinoma, the expression of cyclin D1 is positively correlated with CDC25B (r=0.392, P<0.05).

Above all, the expression of cyclin D1, p27, and CDC25B in gastric cancer tissues was higher and has correlation each other. Detecting of three is providing a new method for the prevention and treatment of gastric cancer.

CONCLUSION

Expression of cyclin D1, CDC25B, and p27 protein can be helpful in the prediction of the biological behavior and prognosis of gastric carcinoma.

REFERENCES

1. Sallinen SL, Sallinen PK, Kononen JT, Syrjäkoski KM, Nupponen NN, Rantala IS, et al. Cyclin D1 expression in astrocytomas is associated with cell proliferation activity and patient prognosis. Pathology. 1999;188(3):289-293.
   http://doi.org/10.1002/(SICI)1096-9896(199907)188:3<289:AID-PATH351>3.0.CO;2-X

2. Drummond S, Canavarro C, Perinetti G, Teles R, Capelli J Jr. The monitoring of gingival crevicular fluid volume during orthodontic treatment a longitudinal randomized split mouth study. J Eur J Orthod. 2012;34(1):109-13.
   http://doi.org/10.1093/ejo/cjq172

3. Hayakawa Y, Hirata Y, Nakagawa H, Sakamoto K, Hikiba Y, Kinoshita H, et al. Apoptosis signal-regulating kinase 1 and cyclin D1 compose a positive feedback loop contributing to tumor growth in gastric cancer. Proc Natl Acad Sci U S A. 2011;108(2):780-785.
   http://doi.org/10.1073/pnas.1011418108

4. Gu HP, Huang Y, Shang PZ. Relationship between the expression of CyclinD1 and p16 protein in human cholangiocarcinomas and its metastasis and prognosis. J Hepatopancreatobiliary Surg. 2013;25(3):219-231.

5. Arber N, Garnmon MD, Hibshoosh H, Britton JA, Zhang Y, Schonberg JB, et al. Overexpression of cyclinD1 occurs in both sguamous carcinomas and adenocarcinomas of the esophagus and in adenocarcinomas of the stomach of the stomach. Hum Pathol. 1999;30(9):1087-1092.
http://doi.org/10.1016/s0046-8177(99)90227-7

6. Bugler B, Quaranta M, Aressy B, Brezak MC, Prevost G and Ducommun B. Genotoxic-activated G2-M checkpoint exit is dependent on CDC25B phosphatase expression. Mol Cancer Ther. 2006;5(6):1446-1451.
http://doi.org/10.1158/1535-7163.MCT-06-0099

7. Yu XY, Zhang Z, Zhang GJ, Guo KF and Kong CZ. Knockdown of Cdc25B in renal cell carcinoma is associated with decreased malignant features. Asian Pac J Cancer Prev. 2012;13(3):931-935.
http://doi.org/10.7314/apjcp.2012.13.3.931.

8. Namagerdi AA, d'Angelo D, Ciani F, Iannuzzi CA, Napolitano F, Avallone L, et al. Triple-negative breast cancer comparison with canine mammary tumors from light microscopy to molecular pathology. Front Oncol. 2020;10:563779.
https://doi.org/10.3389/fonc.2020.563779

9. Xiong DD, He RQ, Lan AH, Chen WJ, Luo YH, Ye ZH, et al. Clinical significances of p27 in digestive tract cancers: a comprehensive analysis on immunohistochemistry staining, published literatures, microarray and RNA-seq data. Oncotarget. 2018; 9(15): 12284–12303.
http//doi.org/10.18632/oncotarget.24316

10. Nishioka K, Doki Y, Shiozaki H, Yamamoto H, Tamura S, Yasuda T, et al. Clinical significance of CDC25A and CDC25B e xpress ion in squamous cell carcinomas of the oesophagus. Br J Cancer. 2001;85(3):412-421.
http://doi.org/10.1054/bjoc.2001.1934

11. Ru Y, Chen XJ, Zhao ZW, Zhang PF, Feng SH, Gao Q, et al. CyclinD1 and p57kip2 as biomarkers in differentiation, metastasis and prognosis of gastric cardia adenocarcinoma. Oncotarget. 2017;8(43):73860-73870.
http://doi.org/10.18632/oncotarget.18008

12. Zhou Z, Zhu JS, Gao CP, Li LP, Zhou C, Wang H, et al. siRNA targeting YAP gene inhibits gastric carcinoma growth and tumor metastasis in SCID mice. Oncol Lett. 2016;11(4):2806-2814.
http://doi.org/10.3892/ol.2016.4319

13. Chaithongyot S, Jantaree P, Sokolova O, Naumann M. NF-κB in gastric cancer development and therapy. Biomedicines. 2021;9(8):870.
http://doi.org/10.3390/biomedicines9080870

14. Du R, Xia L, Sun S, Lian Z, Zou X, Gao J, et al. URG11 promotes gastric cancer growth and invasion by activation of beta-catenin signalling pathway. J Cell Mol Med. 2010;14(3):621-635.
http://doi.org/10.1111/j.1582-4934.2008.00622.x

15. Xu DZ, Geng QR, Tian Y, Cai MY, Fang XJ, Zhan YQ, et al. Activated mammalian target of rapamycin is a potential therapeutic target in gastric cancer. BMC Cancer. 2010;10:536.
http://doi.org/10.1186/1471-2407-10-536

16. Martinson HA, Mallari D, Richter C, Wu TT, Tiesinga J, Alberts SR, et al. Molecular classification of gastric cancer among alaska native people. Cancers (Basel). 2020;12(1):198.
http://doi.org/10.3390/cancers12010198

17. Zhang J, Wang G, Chu SJ, Zhu JS, Zhang R, Lu WW, et al. Loss of large tumor suppressor 1 promotes growth and metastasis of gastric cancer cells through upregulation of the YAP signaling. Oncotarget. 2016;7(13):16180-16193.
http://doi.org/10.18632/oncotarget.7568

18. Chung WC, Zhou Y, Atfi A, Xu K. Downregulation of notch signaling in kras-induced gastric metaplasia. Neoplasia. 2019;21(8):810-821.
http://doi.org/10.1016/j.neo.2019.06.003

19. Suzuki K, Sentani K, Tanaka H, Yano T, Suzuki K, Oshima M, et al. Deficiency of stomach-type claudin-18 in mice induces gastric tumor formation independent of *H. pylori* infection. Cell Mol Gastroenterol Hepatol. 2019;8(1):119-142.
http://doi.org/10.1016/j.jcmgh.2019.03.003

20. Ding L, El Zaatari M, Merchant JL. Recapitulating human gastric cancer pathogenesis: Experimental models of gastric cancer. Adv Exp Med Biol. 2016;908:441-478.
http://doi.org/10.1007/978-3-319-41388-4_22

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