PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

WJG, 1999 December; 5(6):488-491
World Journal of Gastroenterology
 ISSN 1007-9327

The expression of c-src gene in the carcinogenesis process of human cardia adenocarcinoma

WANG Xiu-Jia, YUAN Shu-Lan, XIAO Lin, WANG Xu-Hua and WANG Chao-Jun

Subject headings c-src gene; expression product; PP60$^{c\text{-}src}$; cardia adenocarcinoma; carcinogenesis; neoplasm metastasis; immunohi stochemistry

Abstract

AIM To investigate the activation, expression of c-src gene and its role in the carcinogenetic process of human cardia adenocarcinoma (CA).

METHODS Fifty-six cases of CA, 34 cases of normal, 36 cases of protife rative epithelia adjacent to carcinoma, and 20 cases of lymph node metastases of CA were studied for PP60$^{c\text{-}src}$, the expression product of *c-src* gene immunohistochemically by using the specific monoclonal antibody, Mab327.

RESULTS The positive rates of PP60$^{c\text{-}src}$ in the normal ep ithelia, protiferative epithelia, CA and lymph node metastases were 29.4% (10/ 34), 94.4% (34/36), 71.4% (40/56) and 60.0% (12/20), respectively, among them, the differences of the positive rates were statistically significant ($P < 0.01$). The expression levels of PP60$^{c\text{-}src}$ in CA and proliferative epithe lia were significantly higher than that in the normal epithelia ($P<0.01$) . The PP60$^{c\text{-}src}$ positive rates in the papillary, tubular, poorly differentiated and mucous adenocarcinoma were 75.0% (6/8), 81.8% (18/22), 50.0% (10/20) and 100.0% (6/6), respectively, whereas those of tubular and mucous adenocarcinomas were significantly higher than those of papillary and poorly differentiated adenocarcinomas ($P<0.05$), and the PP60$^{c\text{-}src}$ expression levels of tubular and mucous adenocarcinomas were also significantly higher than those of papillary and poorly differentiated adenocarci nomas($P<0.01$).

CONCLUSION The activation and expression of c-src gene are associated with the initiation and development of human CA; the protein amount of PP60$^{c\text{-}src}$ increased during the process of carcinogenesis; and PP60$^{c\text{-}src}$ expression is also related to lymph node metastases.

INTRODUCTION

There is a general tendency in gastric cancer that the incidence rate of cardia adenocarcinoma (CA) is increasing steadly, and cancer of the distal stomach is decreasing proportionately. The biological and epidemiological features of CA are distinct from those of the distal stomach, and the underlying cause remained unelucidated[1-3]. PP60$^{c\text{-}src}$ is the product of $^{c\text{-}src}$ gene pos sessing the activity of tyrosine kinase. Increased expression of$^{c\text{-}src}$ gene had been reported in some human sarcoma and cancers of breast[4], esoph agus[5], stomach[6] and colon[7], and the activation and expression of c-src gene might be associated with the initiation and devel opment of some cancers. Our previous study showed the activation and expression of $^{c\text{-}src}$ gene was associated with the development and differentiation of esophageal squamous cell carcinomas[8], we believed that s imilar changes might occur in cancer of cardia, therefore, the following study was carried out.

MATERIALS AND METHODS

Sample collection and processing

All 56 cases of CA samples were collected from the CA patients surgically treated in Yanting Institute of Cancer Prevention of Sichuan Province. All tissue specimens were routinely processed, formalin-fixed and paraffin-embedded, at least 2 serial paraffin sections of 4 μm - 6 μm thickness were made, one was stained with hematoxylin and eosin (HE) and the other was use d for PP60$^{c\text{-}src}$ protein detection by immunohistochemical staining.

Reagents

The monoclonal antibody Mab327 (mouse IgG) was kindly given by the Molecular Pathology

Institute of Cancer Research, Cancer Center, The First University Hospital of West China University of Medical Sciences, Chengdu 610041, Sichuan Pro vince, China
WANG Xiu-Jie, male, born on 1957-02-15 in Ziyang, Sichuan Province and graduated from West China University of Medical Sciences in 1982, now Associat e Professor of Oncology, engaged in the researches of etiology and mechanisms of carcinogenesis of cancers, screening and developing anti-cancer drugs, having 20 papers published.
Project supported by the grant of West China University of Medical Scien ces, No.L293015
Correspondence to: WANG Xiu-Jie, Institute of Cancer Research, Cancer Center, The First University Hospital of West China University of Medical Sciences, Chengdu 610041, Sichuan Province, China.
Tel. +86·28·5501218, Fax. +86·28·5583252
Received 1999-07-21 **Accepted** 1999-09-22

Department of Nagoya University, Japan; Streptavidin-Peroxidase Immunohistochemical Staining Kit (Zymed USA) was purchased from Fuzhou Maxim Biotech, Inc.

Immunohistochemical analysis of PP60$^{c\text{-}src}$ protein

PP60$^{c\text{-}src}$ protein was detected immunohistochemically with LSAB method according to the manufacturer's instructions with slight modification. Briefly, the tissue sections were deparaffinized and rehydrated through graded alcohols, and digested with trypsin. Then, endogenous peroxidase activity was blocked with 3% H_2O_2, and after treatment with normal serum, the sections were incubate d with Mab 327 at a dilution of 1:100 overnight at 4℃, with biotinylated second antibody 20 min, and with streptavidin peroxides 30 min at room temperature. Subsequently, the sections were subjected to color reaction with 0.02% 3, 3-diaminobenzidine tetrahydrochloride containing 0.005% H_2O_2 in PBS (pH 7.4), and were counterstained with hematoxylin lightly. In each staining run, a known PP60$^{c\text{-}src}$ positive sample was added as positive control, and a section of the same sample was incubated with PBS instead of Mab327 as negative control.

Histopathological examination

Histopathological diagnosis for CA and related lesions were made, and the histological types of CA were examined by 2 expierenced pathologists according to the given criteria[8].

Qualitative and quantitative analysis of immunohistochemical staining o f PP60$^{c\text{-}src}$

The immunostaining results of PP60$^{c\text{-}src}$ were analyzed according to the kn own criteria[5]. The percentages of positive PP60$^{c\text{-}src}$ cell in CA and related lesions were assessed and scored as follows: negative (-), <25% (+), 25%-50% (++), >50% (+++); The intensity of staining in PP60$^{c\text{-}src}$ positive cytoplasm and/or cell membrane were compared with the negative control and scored as follows: negative (-), weak (+), moderate (++) and strong (+++).

RESULTS

Histopathologic examination

Among the 56 cases of CA samples, there were 34 normal epithelia adjacent to cancer, 36 proliferative epithelia adjacent to cancer, 56 adenocarcinomas including papillary (8/56), tubular (22/56), poorly differentiated (20/56), mucous adenocarcinomas (6/56), and 20 CA with lymph node metastases.

Localization and distribution of PP60$^{c\text{-}src}$

Positive PP60$^{c\text{-}src}$ protein cells showed brown staining in their cytoplasm s and cell membranes, no positive staining was found in the negative controls. The intensity of staining varied with different lesions and different histologic types. Positive staining of PP60$^{c\text{-}src}$ was localized in apex of glandular epithelial cells (Figure 1); in papillary and tubular adenocarcinomas, positive staining was distributed along the papillary margin or glandular lining, both the cytoplasms and cell membranes were positively stained but that of cell membranes were stronger than those in cytoplasms (Figure 2); the positive staining in poorly differentiated adenocarcinomas were evenly distributed in cytoplasms and cell membranes (Figure 3); and in the mucous adenocarcinomas, the positive PP60$^{c\text{-}src}$ staining was unevenly distributed as micromasses.

PP60$^{c\text{-}src}$ expression in CA and in the related lesions

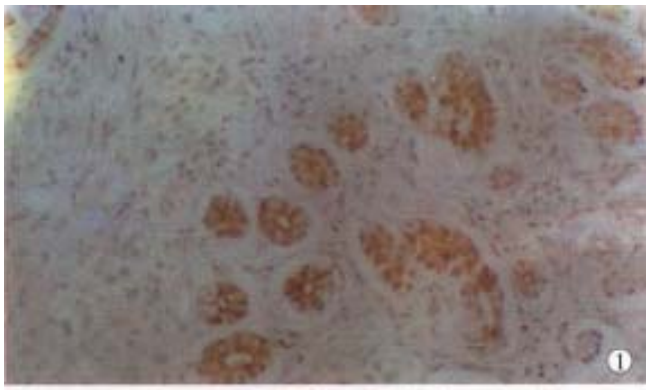
The positive rates of PP60$^{c\text{-}src}$ expression in CA, proliferative and norma l epithelia adjacent to cancer were 71.4% (40/56), 94.4% (34/36) and 29.4% (10/34), respectively (Table 1). The positive rates of the former two were higher than that in the latter (P<0.01), and the positive staining in tensities in CA and proliferative epithelia were also stronger than that in the normal epithelia (P<0.01).

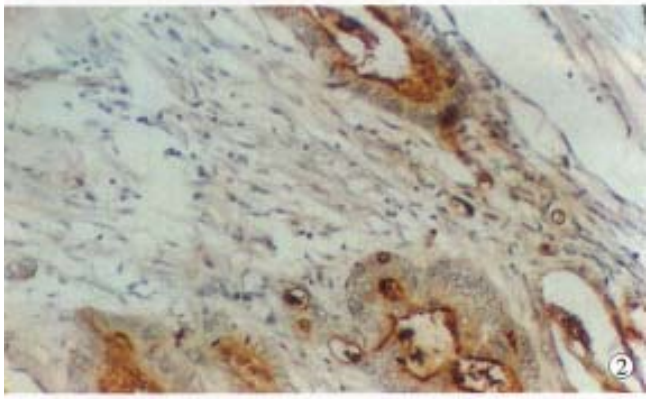
PP60$^{c\text{-}src}$ expression in different histological types

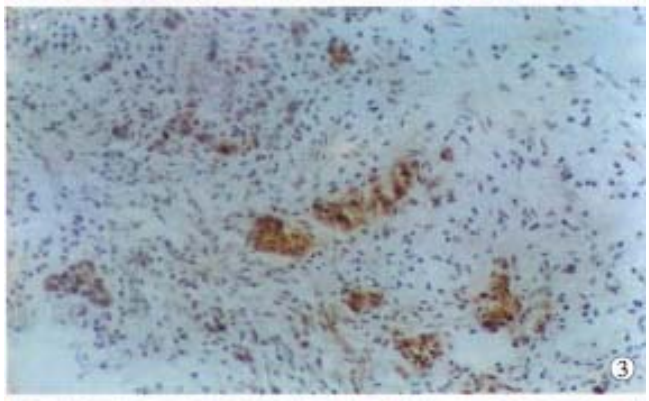
The positive rates of PP60$^{c\text{-}src}$ in papillary, tubular, poorly differentia ted and mucous adenocarcinoma were 75.0% (6/8), 81.8% (18/22), 50.0% (10/20), and 100.0% (6/6), respectively (Table 2) and the positive rates in tubular and mucous adenocarcinomas were higher than those in poorly differentiated or papillary adenocarcinomas (P <0.05); the percentages of high PP60$^{c\text{-}src^{aa}}$ expression (+ + - + + +) in tubular and mucous adenocarcinoma were 63.6% (14/24) and 100.0% (6/6), respectively; while that in papillary and poorly differentiated ones were all low (+), there were significant differences between them (P<0.01).

PP60$^{c\text{-}src}$ expression in CA with lymph node metastases

The positive rate of PP60$^{c\text{-}src}$ expression was 60.0% (12/20) in 20 case s of metastatic lymph node (Table 1), among them, the positive ones in papillary, tubular, poorly differentiated and mucous adenocarcinomas were 2/2, 2/4, 6/12 and 2/2, respectively; the expression intensity of P P60$^{c\text{-}src}$ in CA with lymph node metastases was the same as those without (Figure 4).







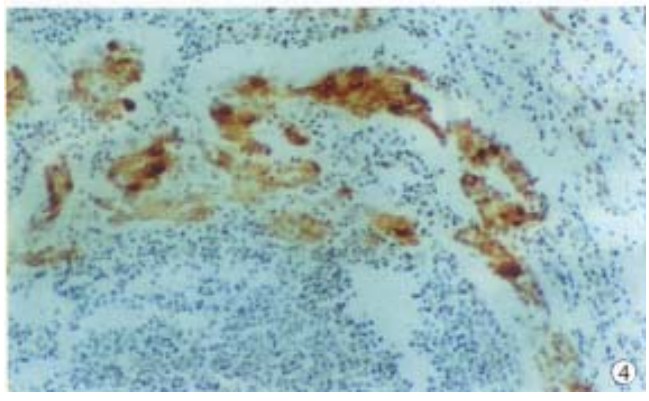


Figure 1 The expression of PP60$^{c\text{-}src}$ in the p roliferative epithelia adjacent to carcinoma. LSAB×200

Figure 2 The expression of PP60$^{c\text{-}src}$ in tubular adenocarcinoma. LSAB×200

Figure 3 The expression of PP60$^{c\text{-}src}$ in the poorly differentiated adenocarcinoma. LSAB×200

Figure 4 The expression of PP60$^{c\text{-}src}$ in the lymph node metastasis of CA. LSAB×200

Table 1 The expression of PP60$^{c\text{-}src}$ in CA and related lesions

Lesion	Case	Staining intensity			Positive rate
		-(%)	+(%)	+ +-+ + +(%)	(%)
Normal epithelia	34	24(70.5)	10(29.4)	0(0)	10(29.4)
Proliferative epithelia	36	2 (5.6)	6(16.7)	28(77.8)[d]	34(94.4)[b]
Cardia adenocarcinoma	56	16(28.6)	20(35.7)	20(35.7)[d]	40(71.4)[b]
Lymph node metastases	20	8(40.0)	8(40.0)	4(20.0)	12(60.0)

[b]P<0.01, in comparison of the positive rates in different lesions (χ^2=34.19) *vs* normal epithelia; [d]P<0.01, in comparison of the positive intensity in different lesions (χ^2 = 25.08) *vs* normal epithelia.

Table 2 The expression of PP60$^{c\text{-}src}$ in CA of different histo logical types

Lesion	Case	Staining intensity			Positive rate
		-(0/10)	+(%)	+ +-+ + +(%)	(%)
Papillary	8	2(25.0)	6(75.0)	0(0)	6(75.0)
Tubular	22	4(18.2)	4(18.2)	14(63.6)[b]	18(81.8)[a]
Poorly differentiated	20	10(50.0)	10(50.0)	0(0)	10(50.0)
Mucous	6	0(0)	0(0)	6(100.0)[b]	6(100.0)[a]
Total	56	16(28.6)	20(35.7)	20(35.7)	40(71.4)

[a]P<0.05,in comparison of the positive rat es of tubular and mucous adenocarcinomas *vs* those of papillary and poorly differentiated adenocarcinomas (χ^2 = 8.11); [b]P<0.01,in comparison of the positive rates of tubular and mucous adenocarcinomas *vs* those of papillary and poorly differentiated adenocarcinomas (χ^2 = 27.56).

DISCUSSION

PP60$^{c\text{-}src}$ is a phosphorylated cytoplasmic protein encoded by c-src gene, having the activity of tyrosine kinase. The expression of PP60$^{c\text{-}src}$ can be found in many normal cells, which plays important roles in the regulation of cell proliferation, differentiation and transformation[4] Recent studies indicated that the increase in the amount of PP60$^{c\text{-}src}$ protein and kinase activity was associated with initiation and development of some human neoplasms, and the activation and increase of expression were one of the factors for cancer initiation[4-8]. In this study, the expressions of PP60$^{c\text{-}src}$ in CA, proliferative epithelia and normal epithelia were 71.4% (40/56), 94.4% (34/36) and 29.4% (10/34), respectively and the positive rates of PP60 $^{c\text{-}src}$ in CA and proliferative epithelia were much higher than that in the normal epithelia (P<0.01). Jankowiski *et al* analyzed the expression product of c-src gene in 15 cases of esophageal adenocarcinoma and 15 cases of Barrett's esophageal epithelia immunohistochemically, the positive rates were 20% (3/15), suggestin that the expression of *c-src* gene is related to the development of esophageal adenocarcinoma. Therefore, the results of t his study indicated that the activation and expression of c-src gene might be associated with the initiation and development of CA. However, the high expre ssion of PP60$^{c\text{-}src}$ in the proliferative epithelia might be associated with the proliferation of glandular epithelial cells, occurring in the aged rats[9]. The low PP60$^{c\text{-}src}$ expression in some normal epithelia adjacent to cancer might be explained by the fact that there had

been PP60$^{c\text{-}src}$ expression in the normal epithelia related to the initiation of cancer[5-8]. On the other h and, it indicated that the activation and expression of c-src gene might be an early event in the carcinogenesis of CA.

Athough it was well known that the activation and expression of *c-src* gene were associated with the initiation of some human neoplasms. The results obtained mainly from biochemical assay by measuring the protein amount and kinase activity of PP60$^{c\text{-}src}$ varied with the methodology. Most of the studies reported that PP60$^{c\text{-}src}$ protein kinase activity increased in the cancer cell lines and cancer tissues from cancers of stomach, colon, lung and kidney, etc., but compared with those of normal tissues related to cancer, no difference of PP60$^{c\text{-}src}$ protein amount was found, the increase in PP60$^{c\text{-}src}$ protein kinase activity could not be explained by the increase of protei n expression encoded by *c-src* gene[6,10]. In the present study, PP60$^{c\text{-}src}$ protein was detected by using the specific monoclonal antibody Mab 327, immunohistochemically, the high level of PP60$^{c\text{-}src}$ expressions (+ + - + + +) in CA and proliferative epithelia were 35.7% and 77.8%, respectively, a low PP60$^{c\text{-}src}$ expression (+) was found in some normal epithelia adjacent to cancer, the difference of expression intensity was significant statistically (P<0.01). The results of this study suggested that the protein amount of PP60$^{c\text{-}src}$ expression was increased in carcinogenesis of CA.

With regard to the relationship between expression product of c-src gene, PP60$^{c\text{-}src}$ and the differentiation of cancer cells, there was no consensus in this aspect[6,8,9,11]. Fanning *et al*[1] reported a high l evel of c-src expression in well differentiated bladder cancers, and pro posed that PP60$^{c\text{-}src}$ protein and kinase activity were associated with the differentiation of epithelial cells of urinary tract and grade I-II bladder carcinomas. However, Takekura *et al*[6] could not find the differen ce of PP60$^{c\text{-}src}$ kinase activity between well and poorly differentiated ga stric carcinomas. In this study, PP60$^{c\text{-}src}$ positive rates varied in different histological types of CA; the positive rates in mucous adenocarcinomas (100.0%, 6/6) and tubular adenocarcinomas (81.8%, 18/22) were higher than those in papillary (75.0%, 6/8) and poorly differentiated types (50.0%, 10/20), the difference being significant statistically (P<0.05). And the high level of expressions (++ - +++) in mucous and tubular adenocarcinomas were 100.0% (6/6) and 63.6% (14/18), respectively, only low expression (+) was found in papillary and poorly differentiated adenocarcinomas, the differences of expression level were also significant statistically (P<0.01). These results suggested that the expression level of PP60$^{c\text{-}src}$ was associated with the differ entiation and histological types of CA, high PP60$^{c\text{-}src}$ expressions in mucous and tubular adenocarcinomas might be related to their well differentiation and other biologic behaviors.

The relationship between the PP60$^{c\text{-}src}$ expression with increase in kinase activity and metastatic colon carcinomas had been reported already[12,13], but there was no report of detection of PP60$^{c\text{-}src}$ protein in lymph no de metastases by immunohistochemical staining. Talamonti *et al*[12] discovered increase in PP60$^{c\text{-}src}$ kinase activity, in the stages o f polyps and primary carcinomas. Termuhlen *et al*[13] reported that P P60$^{c\text{-}src}$ kinase activity in hepatic metastases of colorectal carcinoma in creased by 2.2 folds, and in extrahepatic metastases increased by 12.7 folds, while compared with that in normal mucosa, PP60$^{c\text{-}src}$ kinase activity in the hepatic metastases of non-colorectal carcinomas increased only slightly. In this study, PP60$^{c\text{-}src}$ protein was detected in the lymph node metastases o f CA, the positive rate was 60.0% (12/20), the expression level of PP60$^{c\text{-}src}$ was equal to that of the same primary adenocarcinoma. The results of this study suggested that PP60$^{c\text{-}src}$ expression is associated with the lymph node metastases of CA, which deserved further investigation.

REFERENCES

1 Powell J, McConkey CC. Increasing incidence of adenocarcinoma of the gastric cardia and adjacent sites. *Br J Cancer*, 1990;62:440-443
2 Blot WJ, Devesa SS, Kneller RW, Fraumeni JF. Rising incidence of adenocarcinoma of the esophagus and gastric cardia.*JAMA*, 1991;265:1287-1289
3 Sampliner RE. Adenocarcinoma of the esophagus and gastric cardia: is there progress in the face of increasing cancer incidence.*Ann Intern Med*,1999;130:67-69
4 Jacobs C, Rübsamen H. Expression of PP60 c-src protein kinase in adult and fetal human tissue: high activities in some sarcomas and mammary carcinomas. *Cancer Res*, 1983;43:1696-1702
5 Jankowski J, Coghill G, Hopwood D, Wormely KG. Oncogenes and onco suppressor genes in adenocarcinoma of the esophagus. *Gut*, 1992;33:1033-1038
6 Takekura N, Yasui W, Yoshida K, Tsujino T, Nakayama H, Kameda T, Yokozaki H, Nishimura Y, Ito H, Tahara E. PP60 c-src protein kinase activity in human gastric carcinomas. *Int J Cancer*, 1990;45:847-851
7 Cartwright CA, Kamps MP, Meislei AI, Pipas JM, Eckert W.PP60$^{c\text{-}src}$ activation in human colon carcinoma. *J Clin Invest*,1989;83:2025-2033
8 Wang XJ, Wang CJ, Huang GQ, Xiao HY. A study of c-src gene express product PP60$^{c\text{-}src}$ in esophageal carcinoma.*J WCUMS*, 1995;26:197-201
9 Majumdar APN, Tureaud J, Relan NK, Kessel A, Dutta S, Hatfield JS, Fligiel SEG. Increased expression of PP60$^{c\text{-}src}$ in gastric mucosa of aged rats. *J Gerontol*, 1994;49:B110-116
10 Cartwright CA, Meisler AI, Eckhart W. Activation of the PP60$^{c\text{-}src}$ protein kinase is an early event in colonic carcinogenesis. *Proc Natl Acad Sci USA*, 1990;87:558-562
11 Fanning P, Bulovas K, Saini KS, Libertino JA, Joyce AD, Summerhayes IC. Elevated expression of PP60 c-src in low grade human bladder carcinomas. *Cancer Res*, 1992;52:1457-1462
12 Talamonti MS, Roh MS, Curley SA, Gallick GE. Increase in activity and level of PP60$^{c\text{-}src}$ in progressive stages of human colorectal cancer.*J Clin Invest*, 1993;91:53-60
13 Termuhlen PM, Curley SA, Talamonti MS, Saboorian MH, Gallick GE. Site specific differences in PP60$^{c\text{-}src}$ activity in human colorectal metastases. *J Surg Res*, 1993;54:293-298

Edited by WU Xie-Ning
Proofread by MIAO Qi-Hong