

• BRIEF REPORTS •

## ***c-src* activating mutation analysis in Chinese patients with colorectal cancer**

Ye-Xiong Tan, Han-Tao Wang, Peng Zhang, Zhong-Hua Yan, Guan-Long Dai, Meng-Chao Wu, Hong-Yang Wang

Ye-Xiong Tan, Peng Zhang, Zhong-Hua Yan, Meng-Chao Wu, Hong-Yang Wang, International Co-operational Laboratory on Signal Transduction, Eastern Hepatobiliary Surgery Hospital, Shanghai 200438, China

Han-Tao Wang, Guan-Long Dai, Department of General Surgery, Changhai Hospital, Shanghai 200433, China

Supported by the Major State Basic Research Development Program of China, No. G1998051210

Correspondence to: Dr. Hong-Yang Wang, International Co-operational Laboratory on Signal Transduction, Eastern Hepatobiliary Institute, Shanghai 200438, China. hywangk@online.sh.cn

Telephone: +86-21-25070856 Fax: +86-21-65566851

Received: 2004-04-24 Accepted: 2004-04-29

### **Abstract**

**AIM:** To investigate the occurrence of cellular *src* (*c-src*) activating mutation at codon 531 in colorectal cancer patients from Chinese mainland.

**METHODS:** Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay followed by sequencing and single-strand conformation polymorphism analysis were carried out to screen 110 samples of primary colorectal cancer and 20 colorectal liver metastases.

**RESULTS:** Only one sample showed PCR-RFLP-positive results and carried somatic codon 531 mutations. No additional mutation of *c-src* exon 12 was found.

**CONCLUSION:** *c-src* codon 531 mutation in colorectal cancer is not the cause of *c-src* activation.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

**Key words:** *c-src*; Colorectal cancer

Tan YX, Wang HT, Zhang P, Yan ZH, Dai GL, Wu MC, Wang HY. *c-src* activating mutation analysis in Chinese patients with colorectal cancer. *World J Gastroenterol* 2005; 11(15): 2351-2353

<http://www.wjgnet.com/1007-9327/11/2351.asp>

### **INTRODUCTION**

Liver metastasis is one of the main causes of death of colorectal cancer patients. Although it is well known that tumor metastasis is a multi-factorial and multi-step process, the exact mechanisms underlying metastasis of colorectal cancer are still largely unclear. Elevated expression and activity of the *src* family tyrosine kinase are often associated

with progression and metastasis of human colorectal cancer<sup>[1]</sup>. As for the mechanism of *src* activation, Irby *et al*<sup>[2]</sup>, reported an activating *c-src* mutation, C-T mutation in codon 531 (located at exon 12), in 12% of advanced human colon cancers<sup>[2]</sup>. But the following likewise screens of this kind of mutations in four groups all had negative results<sup>[3-6]</sup>. It is still quite uncertain whether codon 531 activating mutation is a cause of *c-src* activation in advanced colorectal cancers.

In this study, we investigated 110 primary colorectal cancers and 20 colorectal liver metastasis patients from China mainland for this *c-src* specific mutation.

### **MATERIALS AND METHODS**

#### **Tissue specimens**

Primary colorectal tumors and corresponding normal tissues and blood samples were obtained from a total of 110 Chinese patients (median age of 52 years, ranging from 36 to 72 years) with primary colorectal cancer during surgery at Changhai Hospital and Changzheng Hospital (both in Shanghai, China) from May 2003 to December 2003. The tumors were classified as Duke stage A in 21, B in 26, C in 38 and D in 25. Twenty liver metastases with colorectal origin were from Shanghai Eastern Hepatobiliary Surgery Hospital. Tissue samples were stored at -80 °C until DNA extraction.

#### **PCR amplification and RFLP analysis**

Genomic DNA was extracted as previously described<sup>[7]</sup>. DNAs from all 130 samples served as templates for PCR (Perkin-Elmer) with primers set to amplify exon 12 of *c-src*. The sequences of primers were as follows: forward, 5'-ACAGGGATGGTGAACCGCGA-3' and reverse, 5'-ATCCAAGCCGAGAAGCCGGT-3'.

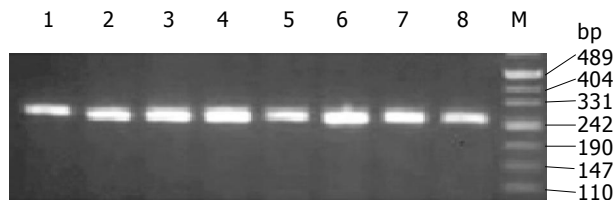
After DNA extraction (QIAquick PCR purification kit, Qiagen), PCR products were digested with 5 Units of restriction enzyme *Sca* I overnight. Plasmid DNAs bearing *src* 531 mutation created by using overlap extension PCR<sup>[8]</sup> were used as positive controls. The possible digestion-positive samples were cloned into T-vector (pGEM®-T vector, Promega) and sequenced automatically (Perkin Elmer-Roche, Banchburg, NJ).

#### **SSCP analysis**

PCR products of genomic DNA were screened for mutations by single-strand conformational polymorphism (SSCP) analysis exactly as previously described<sup>[9]</sup>.

### **RESULTS**

PCR with primers devised here generated a 266-bp fragment,



**Figure 1** PCR amplification of *src* exon 12 from genomic DNA of colorectal cancer. Lanes 1-8: samples; lane M: marker.

which was confirmed to be exon 12 of *c-src* by direct sequencing (Figure 1). Only one sample (Figure 2) was positive by RFLP analysis among the 110 samples of colorectal cancer and 20 liver metastases in this study. This No. 73 sample could only be partially digested by 5U *Sca* I even after overnight digestion, implying that the corresponding DNA template was a mixture of mutated and wild type clones.

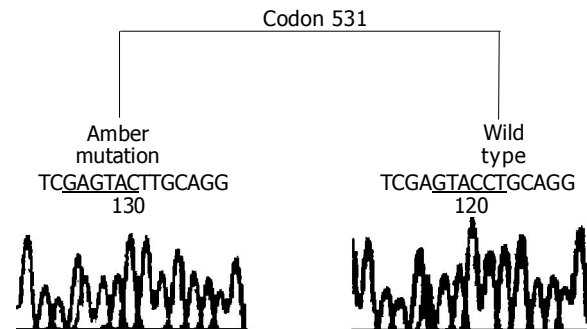
This positive PCR product was cloned into T vector, and 20 transformants were selected for sequencing. The result confirmed the presence of 531 C-T mutation and the ratio of mutated clones *vs* wild type clones was 8:12 (Figure 3). To further know whether it was a somatic or germ line mutation, we analyzed its paired normal samples and blood likewise, but could not find any mutation (Figure 2).

Additionally, SSCP was carried out to screen possible other mutations of *c-src* in exon 12. No aberrant band was found except for No. 73 sample and positive control (Figure 4).

## DISCUSSION

Cellular *src* (*c-src*) is a human homolog of the Rous sarcoma viral oncogene, *v-src*. *c-src* has been implicated in the development and progression of numerous human cancers, including colorectal cancer<sup>[10]</sup>. *c-src* was shown to be dramatically activated in colorectal cancer<sup>[11,12]</sup>. Irby *et al*<sup>[2]</sup>, reported *src* point mutation at codon 531 truncated *c-src* C-terminal to the negatively regulatory kinase phosphorylation site at Tyr 530 and thereby activated *c-src*. This was the first report of activating *c-src* mutation related to the progression of colorectal cancer. However, no such mutations in Japanese and North European as well as from Italian patients with colorectal cancer were found<sup>[3-6]</sup>.

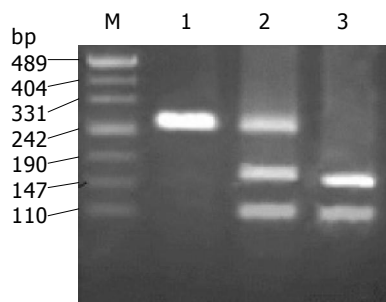
In this research, 110 primary colorectal cancers (38 in



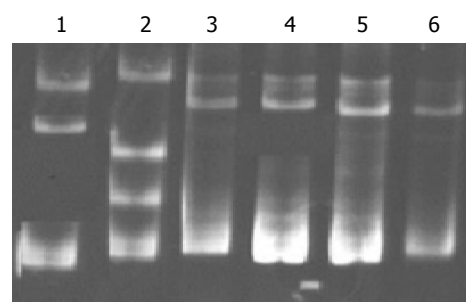
**Figure 3** Sequencing of two clones (one wild and the other Amber-mutated) from RFLP-positive PCR products of No. 73 colorectal cancer.

Duke's stage C and 25 in Duke's stage D) and 20 liver metastases were screened by RFLP analysis. Only one sample showed positive results (Figure 2) and was confirmed by direct sequencing, while its adjacent normal intestinal mucosa and blood kept intact. Our results indicated that activating mutation of *c-src* might be only a rare case, which could not explain the *c-src* activation always found in colorectal cancer.

*c-src* can be activated through various mechanisms, the core of which is to relieve the intramolecular autoinhibition created by binding of its SH2 domain to pY527 in the carboxyl terminus<sup>[13]</sup>. pY527 is phosphorylated by the protein tyrosine kinase, Csk. Recently, Cam *et al*<sup>[14]</sup>, found that Csk protein and its kinase activity were reduced in colorectal carcinoma and correlated with *c-src* kinase activity. Furthermore, Csk over-expression in mouse NL-17 cells (highly metastatic clone of mouse colon adenocarcinoma) resulted in significant suppression of metastasis, but did not affect its tumorigenicity<sup>[15]</sup>, demonstrating its role in metastatic inhibition. More recently, Rengifo-Cam *et al*<sup>[16]</sup>, found that overexpression of wild-type Csk increased cell-cell contacts, mediated by E-cadherin, could decrease the number of focal contacts and cell adhesion/migration and *in vitro* invasiveness. It is, therefore, worthwhile to detect the activity of Csk in colorectal cancer. However, Be'nistant *et al*<sup>[17]</sup>, reported the existence of autoantibodies to Csk and elevated Csk in colorectal adenocarcinoma. Furthermore, it was found that *src* was deregulated in all of the tumors tested, suggesting that Csk could not phosphorylate *src* in transformed cells or that *src* was activated despite its phospho-



**Figure 2** Positive result of No. 73 sample in RFLP analysis. Lane 1: No. 73 para-cancer tissue; lane 2: No. 73 cancer tissue; lane 3: positive control; lane M: DNA marker.



**Figure 4** SSCP analysis of *src* exon 12 fragment amplified from colorectal cancer. Lane 1: positive control; lane 2: No. 73; lane 3: negative control; lanes 4-6: three negative samples.

orylation by Csk.

Apart from down-regulation of csk, transcriptional activation might be another mechanism of *c-src* activation in advanced colorectal cancer<sup>[18]</sup>. Our study demonstrates that activating point mutation in codon 531 may not be the main mechanism of *c-src* activation in colorectal cancer.

## REFERENCES

- 1 **Yeatman TJ.** A renaissance for SRC. *Nat Rev Cancer* 2004; **4**: 470-480
- 2 **Irby RB,** Mao W, Coppola D, Kang J, Loubeau JM, Trudeau W, Karl R, Fujita DJ, Jove R, Yeatman TJ. Activating SRC mutation in a subset of advanced human colon cancers. *Nat Genet* 1999; **21**: 187-191
- 3 **Daigo Y,** Furukawa Y, Kawasoe T, Ishiguro H, Fujita M, Sugai S, Nakamori S, Liefers GJ, Tollenaar RA, van de Velde CJ, Nakamura Y. Absence of genetic alteration at codon 531 of the human *c-src* gene in 479 advanced colorectal cancers from Japanese and Caucasian patients. *Cancer Res* 1999; **59**: 4222-4224
- 4 **Nilbert M,** Fernebro E. Lack of activating *c-SRC* mutations at codon 531 in rectal cancer. *Cancer Genet Cytogenet* 2000; **121**: 94-95
- 5 **Wang NM,** Yeh KT, Tsai CH, Chen SJ, Chang JG. No evidence of correlation between mutation at codon 531 of *src* and the risk of colon cancer in Chinese. *Cancer Lett* 2000; **150**: 201-204
- 6 **Laghi L,** Bianchi P, Orbetegli O, Gennari L, Roncalli M, Malesci A. Lack of mutation at codon 531 of *SRC* in advanced colorectal cancers from Italian patients. *Br J Cancer* 2001; **84**: 196-198
- 7 **Blin N,** Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res* 1976; **3**: 2303-2308
- 8 **Ho SN,** Hunt HD, Horton RM, Pullen JK, Pease LR. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. *Gene* 1989; **77**: 51-59
- 9 **Orita M,** Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 1989; **5**: 874-879
- 10 **Warmuth M,** Damoiseaux R, Liu Y, Fabbro D, Gray N. SRC family kinases: potential targets for the treatment of human cancer and leukemia. *Curr Pharm Des* 2003; **9**: 2043-2059
- 11 **Talamonti MS,** Roh MS, Curley SA, Gallick GE. Increase in activity and level of pp60 *c-src* in progressive stages of human colorectal cancer. *J Clin Invest* 1993; **91**: 53-60
- 12 **Iravani S,** Mao W, Fu L, Karl R, Yeatman T, Jove R, Coppola D. Elevated *c-Src* protein expression is an early event in colonic neoplasia. *Lab Invest* 1998; **78**: 365-371
- 13 **Schlessinger J.** New roles for Src kinases in control of cell survival and angiogenesis. *Cell* 2000; **100**: 293-296
- 14 **Cam WR,** Masaki T, Shiratori Y, Kato N, Ikenoue T, Okamoto M, Igarashi K, Sano T, Omata M. Reduced C-terminal Src kinase activity is correlated inversely with pp60 (*c-src*) activity in colorectal carcinoma. *Cancer* 2001; **92**: 61-70
- 15 **Nakagawa T,** Tanaka S, Suzuki H, Takayanagi H, Miyazaki T, Nakamura K, Tsuruo T. Overexpression of the csk gene suppresses tumor metastasis *in vivo*. *Int J Cancer* 2000; **88**: 384-391
- 16 **Rengifo-Cam W,** Konishi A, Morishita N, Matsuoka H, Yamori T, Nada S, Okada M. Csk defines the ability of integrin-mediated cell adhesion and migration in human colon cancer cells: implication for a potential role in cancer metastasis. *Oncogene* 2004; **23**: 289-297
- 17 **Be'nistant C,** Bourgaux JF, Chapuis H, Mottet N, Roche S, Bali JP. The COOH-terminal Src kinase Csk is a tumor antigen in human carcinoma. *Cancer Res* 2001; **61**: 1415-1420
- 18 **Dehm S,** Senger MA, Bonham K. SRC transcriptional activation in a subset of human colon cancer cell lines. *FEBS Lett* 2001; **487**: 367-371

Science Editor Wang XL and Kumar M Language Editor Elsevier HK