Clin Chem Lab Med 2009;47(7):811–817 © 2009 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2009.186

Expression of *RhoA* and *RhoC* in colorectal carcinoma and its relations with clinicopathological parameters

Hai-bo Wang[1], Xiang-ping Liu[2,*], Jun Liang[3], Kun Yang[2], Ai-hua Sui[2] and Yan-jun Liu[1]

[1] Department of General Surgery, Affiliated Hospital of Qingdao University Medical College, Qingdao, China

[2] Central Laboratory of Molecular Biology, Affiliated Hospital of Qingdao University Medical College, Qingdao, China

[3] Department of Oncology, Affiliated Hospital of Qingdao University Medical College, Qingdao, China

Abstract

Background: Ras homologous (Rho) family GTPases play a pivotal role in the regulation of numerous cellular functions associated with malignant transformation and metastasis. To evaluate the role of these GTPases in colorectal cancer, the mRNA expression levels in matched sets of tumor and non-tumor tissues from surgical specimens were analyzed. The relationship between the mRNA levels in tumor tissues to the clinicopathological features was also assessed.

Methods: A total of 68 patients with colorectal carcinoma were recruited and the levels of *RhoA* and *RhoC* mRNA transcripts in cancer, paratumoral and normal tissues were characterized by quantitative real-time polymerase chain reaction (QRT-PCR). Their correlation to clinical histopathological parameters was analyzed.

Results: The levels of *RhoA* and *RhoC* mRNA transcripts in carcinoma tissues were significantly higher than those in the matched paratumor and normal tissues from the same patient ($p<0.05$). The expression levels of both genes were significantly correlated with metastasis of cancer cells to lymph nodes and liver ($p<0.05$). The levels of *RhoA* expression were significantly correlated with the histopathological degree of cancer, while the expression of *RhoC* was correlated with the extent of local invasion to intestine.

Conclusions: This is the first study with QRT-PCR to examine the expressions of *RhoA* and *RhoC* genes in colorectal carcinoma of Chinese patients. The significantly up-regulated *RhoA* and *RhoC* expressions suggest that they may contribute to the initiation, development, invasion and metastasis of colorectal carcinoma in Chinese patients.
Clin Chem Lab Med 2009;47:811–7.

Keywords: colorectal carcinoma; gene expression; real-time polymerase chain reaction (RT-PCR); *RhoA*; *RhoC*.

Introduction

Colorectal carcinoma is one of the most common malignancies with an increasing annual incidence (1). Colorectal carcinoma is usually accompanied by local invasion and distant metastasis, which are the main causative factors for cancer-related death (2). The initiation, development, local invasion and distal metastasis are closely regulated by multiple genes, whose expression are determined by internal or external factors. Therefore, elucidation of these factors and the pattern of their expression may help to understand the progression of colorectal carcinoma, and help predict the clinical outcome of patients with colorectal carcinoma.

The Ras homologous (Rho) sub-family of low-molecular-weight GTP-binding proteins contains Rho (e.g., *RhoA*, *B*, *C*), Rac and Cdc42 proteins (3). These molecules are involved in the regulation of a variety of cellular processes, such as the organization of the microfilament network, cell–cell contact and malignant transformation (4). Furthermore, they play important roles in intracellular signal transduction, regulate cell proliferation, apoptosis, adhesion and motility (5). Abnormal expression and activation of both *RhoA* and *RhoC* have been demonstrated to correlate with the development and metastasis of malignant carcinoma (3). Increased expression of *RhoC* has been found to correlate with poor outcome in Caucasians with colorectal carcinoma, and may also be used as a prognostic marker in these patients (6). Increased expression of *RhoA* has been observed in Asians with colorectal carcinoma (7). However, little is known about whether the expression of those oncogenes is altered in Chinese patients with colorectal carcinoma, and how this expression is correlated with the clinicopathological characteristics of Chinese patients with colorectal carcinoma.

The quantitative real-time polymerase chain reaction (QRT-PCR) is a new fluorescence-based technology that can be used to amplify and simultaneously quantify targeted DNA molecules. Together with reverse transcription, this technology can be employed to quantitatively measure mRNA transcripts of one gene at a particular time in certain cells

*Corresponding author: Xiang-ping Liu, Associate Professor, Molecular Biology Laboratory, Affiliated Hospital of Qingdao University Medical College, No. 16 Jiangsu Road, Qingdao 266003, China
Phone: +86-532-82911739, Fax: +86-532-82911999,
E-mail: xiangpingliu@126.com
Received January 5, 2009; accepted April 7, 2009; previously published online June 5, 2009

or tissues. In this study, we employed the QRT-PCR to examine the expressions of *RhoA* and *RhoC* genes in colorectal carcinoma tissue from Chinese patients, and analyze the relationship between its expression and various clinicopathological parameters. Our data demonstrated high levels of *RhoA* and *RhoC* mRNA transcripts in colorectal carcinoma from Chinese patients, which may contribute to the initiation, progression and metastasis of colorectal carcinoma.

Materials and methods

Subjects and sample collection

A total of 68 patients with colorectal carcinoma who underwent surgery in the Department of General Surgery, the Affiliated Hospital of Qingdao University Medical College between January 2007 and April 2008, were recruited for this study after informed consent was obtained. There were 38 males and 30 females, with an average age of 61.5 years (range 36–80 years). For the 68 cases, there were 31 with colon carcinoma and 37 with rectal carcinoma. Diagnoses were confirmed by pathological examinations. Prior to surgery, none of the patients received chemotherapy or radiotherapy. At surgery, tumor tissue, paratumor tissue (3–5 cm from the border of the tumor) and normal tissue (>5 cm from the border of the tumor) were resected. Based on the histopathological classifications, six out of 68 cases were highly differentiated, 45 moderately differentiated and 17 low differentiated adenocarcinomas. According to the TNM staging for colorectal carcinoma (UICC, 2006), there were six cases at T_1, 18 at T_2, 35 at T_3 and nine at T_4. Twenty-four patients had metastasis to lymph nodes, and 11 to the liver, including two with concomitant metastasis to the liver and lung. The tissues were immediately frozen in liquid nitrogen and stored at –70°C until use.

Primer design and synthesis

The specific primers used for amplifying the targeted gene fragments with QRT-PCR were designed using the Primer Express software (Applied Biosystems, Foster City, CA, USA), based on the sequences of these genes, and analyzed using the basic local alignment search tool (BLAST) to determine their specificity. The primers for the human *RhoA* gene were: sense 5′-CGGGAGCTAGCCAAGATGAAG-3′, antisense 5′-CCTTGCAGAGCAGCTCTCGTA-3′, fluorescent probe 5′-FAM-AGAGATATGGCAAACAGGATTGGCG-TAMRA-3′, and the amplicon size was 158 base pairs (bp). The primers for the human *RhoC* gene were: sense 5′-CCTCATGTGCTTCTC-CATCGA-3′, antisense 5′-CTCGTCTTGCCTCAGGTCCTT-3′, fluorescent probe 5′-FAM-TCTGCCCCAACGTGCCCATCAT-TAMRA-3′, and the amplicon size was 136 bp. *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) was used as the internal control with the specific primers: sense 5′-CTTAG-CACCCCTGGCCAAG-3′, antisense 5′-GATGTTCTGGAGAGC-CCCG-3′, fluorescent probe 5′-FAM-CATGCCATCACTGCCA-CCCAGAAGA-TAMRA-3′, and the amplicon size was 150 bp. The primers and fluorescent probes were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China).

RNA and cDNA preparation and QRT-PCR

Total RNA was extracted from tissue samples using Trizol (Invitrogen, Gaithersburg, MD, USA) and reverse transcripted into cDNA using the PrimeScript RT-PCR kit (TaKaRa Bio Inc, Shiga, Japan) according to the manufacturer's instructions. To test the specificity of the primers, different sets of primers were tested for amplifying the targeted DNA from the cDNA by polymerase chain reaction (PCR). The reactions were subjected to an initial denaturation at 95°C for 10 s, followed by 40 cycles of denaturation at 95°C for 5 s, and annealing and extension at 60°C for 45 s. The PCR products were analyzed on a 2% agarose gel and by sequencing (Shanghai Sangon, Shanghai, China).

The levels of *RhoA*, *RhoC* and control *GAPDH* mRNA transcripts were determined with the QRT-PCR in an ABI7500 real-time thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR reactions in duplicate were subjected to initial denaturation at 95°C for 10 s, followed by 40 cycles of denaturation at 95°C for 5 s, and annealing and extension at 60°C for 45 s. The value of the threshold cycle (CT) for each reaction was recorded. The levels of *RhoA* and *RhoC* mRNA transcripts relative to *GAPDH* were expressed as the $\Delta CT(CT_{Target}–CT_{GAPDH})$ and $\Delta\Delta CT$, where $\Delta\Delta CT = (CT_{Target}–CT_{GAPDH})_{cancer\ tissue}–(CT_{Target}–CT_{GAPDH})_{normal\ tissue}$ (8). Amplification efficacy of the target and internal control genes were determined, and only the slope value of log target gene copies vs. $\Delta CT < 0.1$ was further quantified using the $2^{-\Delta\Delta CT}$ method. Amplification efficiencies of *RhoA*, *RhoC* and *GAPDH* (internal control) genes were examined by QRT-PCR using serial 2-fold dilutions of reverse-transcribed cDNA. Assays were done in duplicate, and each experiment was repeated 2 times. Data were expressed as mean ± SEM.

Statistical analysis

Data were expressed as mean ± SD. Differences between groups were analyzed using Students t-test or ANOVA with SPSS software (Ver. 11.5, SPSS Inc., Chicago, IL, USA). Correlation between covariates were determined using Pearson correlation analysis. A $p < 0.05$ was used for statistical significance.

Results

Primer specificity

To determine the levels of *RhoA* and *RhoC* mRNA transcripts, total RNA was extracted from individual samples and the purity of these RNA samples determined by the ratio of optical density (OD260/OD280). The ratio was between 1.8 and 2.0, suggesting high purity of RNA. Following reverse transcription into cDNA, the target gene fragments of *RhoA*, *RhoC* and *GAPDH* were amplified by PCR (Figure 1). As expected, individual DNA products were observed with *RhoA* (158 bp), *RhoC* (136 bp) and *GAPDH* (150 bp), respectively (Figure 1), suggesting high-specificity of the primers. Further sequence analysis revealed that the PCR products were *RhoA*, *RhoC* and *GAPDH* DNA fragments (Figure 2). These preliminary results provided a reasonable basis for quantitative characterization of *RhoA*, *RhoC* and *GAPDH* mRNA transcripts in the tissue samples.

Amplification efficiency

Amplification curves of serially diluted cDNA samples exhibited a standard S shape, suggesting a good amplification efficiency and a linear relationship. For

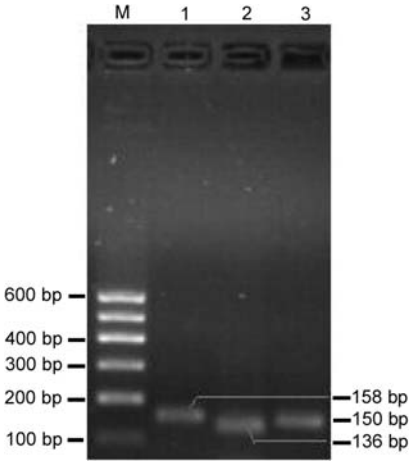

Figure 1 Gel electrophoresis of PCR products. M, size marker; 1, *RhoA*; 2, *RhoC*; 3, *GAPDH*.

the *RhoA* and *RhoC* genes, the log value of each cDNA dilution was plotted vs. ΔCT, showing a slope of 0.0797 and 0.0864, respectively (Figures 3 and 4). This indicated that both target genes had amplification efficiencies similar to the internal control genes (*GAPDH*), justifying application of the $2^{-\Delta\Delta CT}$ method for relative quantification (9).

High levels of *RhoA* and *RhoC* mRNA transcripts in colorectal carcinoma tissues

We first examined the expression of *RhoA, RhoC* and *GAPDH* in tissue samples obtained from 68 patients with colorectal carcinoma (Figure 5). Data analysis showed the values of *RhoA* and *RhoC* mRNA in tumor samples to be significantly higher compared with that found in paratumor or normal tissue ($p<0.05$). The relative levels of *RhoA* and *RhoC* mRNA in tumor samples compared with normal samples were 4.53 (3.81–5.39) and 3.83 (3.11–4.73), respectively. These results demonstrated significantly higher expression of *RhoA* and *RhoC* mRNA in tumor samples ($p<0.05$). However, there was no significant difference between paratumor and normal tissue samples ($p>0.05$) (Table 1). The high levels of *RhoA* and *RhoC* mRNA transcripts shown in colorectal carcinoma tissues suggest that both *RhoA* and *RhoC* expression may contribute to the tumorigenesis of colorectal carcinoma in Chinese patients.

Correlation of *RhoA* and *RhoC* gene expressions to clinical histopathological features of colorectal carcinoma

We analyzed the relationship between the levels of *RhoA* and *RhoC* gene expression. The levels of *RhoA* mRNA transcripts were correlated with that of *RhoC* mRNA expression in the colorectal tumor samples ($r=0.847$, $p<0.01$). Further analysis of the relationship between the levels of *RhoA* or *RhoC* expression and various clinical histopathological features revealed that the expression of both genes was significantly correlated with the presence of metastasis to lymph nodes or liver ($p<0.05$), but was independent of patient's age and gender. In addition, the level of

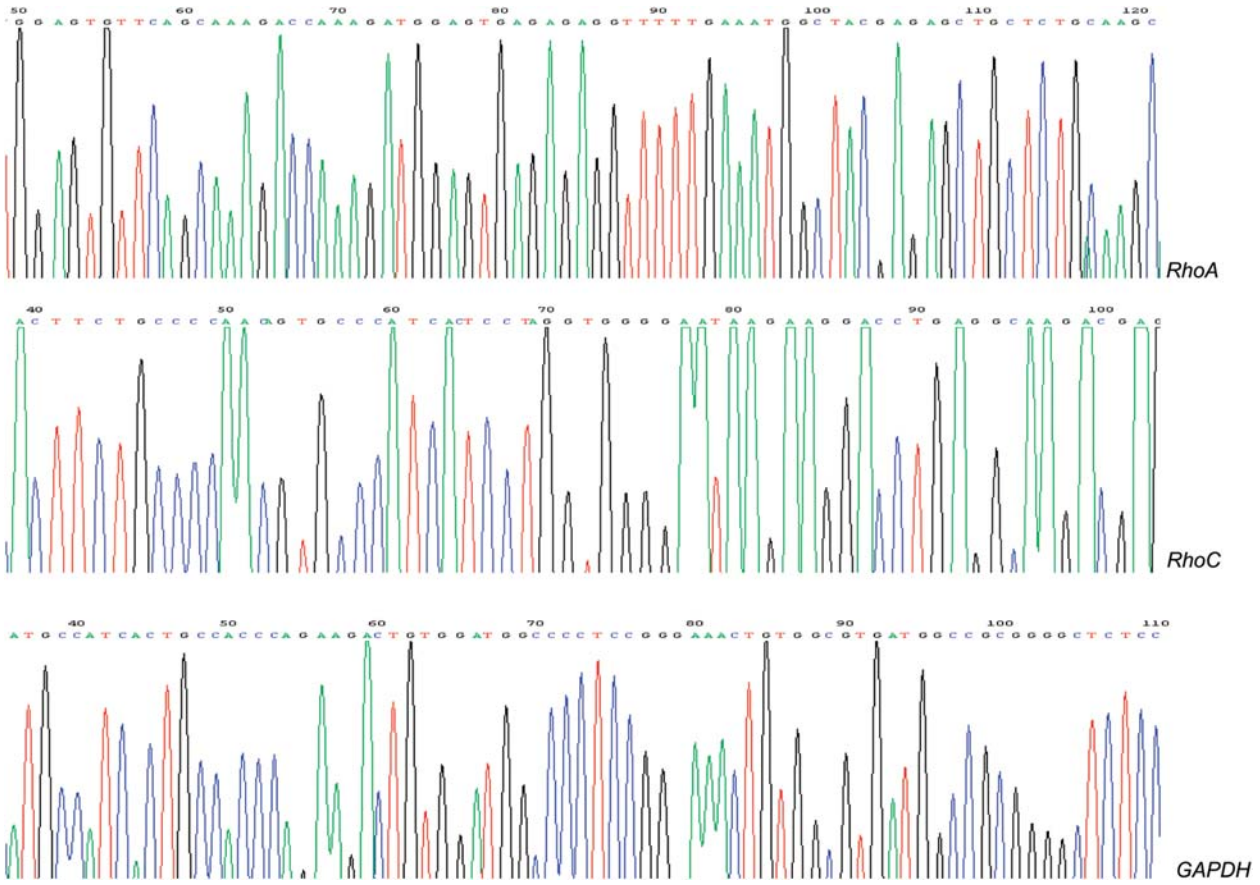

Figure 2 Sequencing histograms of PCR products for *RhoA*, *RhoC* and *GAPDH*.

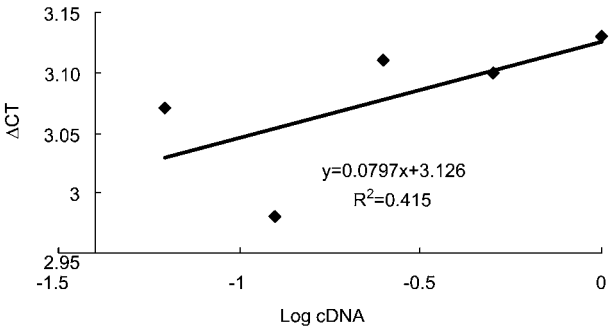

Figure 3 Plot of log input amount vs. $\Delta CT_{(RhoA-GAPDH)}$.

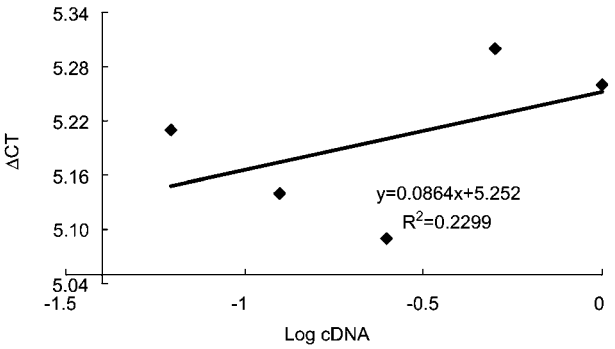

Figure 4 Plot of log input amount vs. $\Delta CT_{(RhoC-GAPDH)}$.

RhoA expression was correlated with histopathological degree of cancer, while *RhoC* was correlated with the extent of local invasion to the intestine ($p < 0.05$) (Table 2).

Discussion

The Rho family of GTPase functions as a molecular switch within cells. In response to intra- and extracellular signals, it cycles between an active GTP-bound form and an inactive GDP-bound form. The Rho family not only regulates the organization of the actin filament system but also modulates cell motility, proliferation, apoptosis, cell cycle progression, and invasion and metastasis of malignant tumor cells (10). Small GTPase of the Rho family plays an important role in eukaryotic signal transduction, which primarily affects the organization of the cytoskeleton involved with movement. Rho-associated protein kinase (ROCK) is a key downstream effector of Rho proteins (11, 12). Recent data have implicated that the Rho/ROCK pathway plays an important role in the invasion and metastasis of cancer cells (13–15).

RhoA has been shown to regulate the activities of multiple transcription factors. The majority of these factors have been implicated in cancer progression (16) by modulating cancer cell adhesion, contraction, movement, release of cellular adhesion, degradation of extra-cellular matrix, and invasion into blood or lymph vessels (17–19). In gastric cancer, high levels of *RhoA* expression were observed in tumors at more advanced stages, histologically diffuse, aggressive metastasis into lymph nodes and poorer survival (20). *RhoA* itself also has transformation activity, often interacting with Ras gene mutations, especially K-ras, which is a common event in colorectal carcinoma, to promote cellular transformation (21).

RhoC also contributes to tumor development, especially invasion and metastasis of cancer cells. Its functions are likely mediated by the following mechanisms (22, 23): 1) disrupting cellular polarity, 2) depriving cells of adherent junctions, 3) promoting cellular motility and remodeling extra-cellular matrix to enhance the local invasiveness of cancer cells, 4) up-regulating the expression of factors involved in angiogenesis to promote the intravasation of tumor cells into blood vessels. *RhoC* has been considered a marker for early metastasis of cancer (24). Clark and colleagues identified high *RhoC* gene expression in highly metastatic melanoma cells by microarray. They were also the first to demonstrate the causative role of *RhoC* expression in tumor cell invasion, proposing the involvement of *RhoC* in tumor invasion and metastasis (25). In prostate cancer, over-expression of *RhoC* enhances cellular invasion, and its

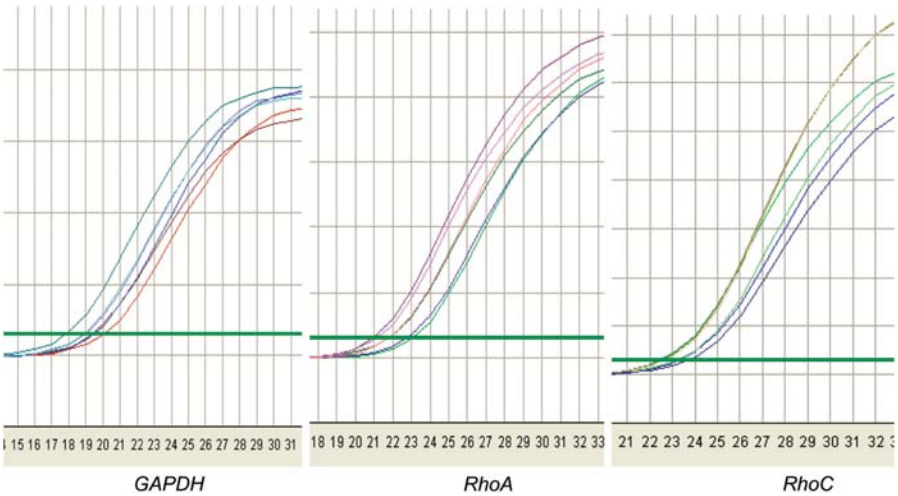

Figure 5 Amplification curve of *GAPDH*, *RhoA* and *RhoC*.

Table 1 Relative expression of *RhoA* and *RhoC* genes in different tissues.

Tissue type	Sample number (n)	*RhoA*		*RhoC*	
		ΔΔCT	Relative to normal[a]	ΔΔCT	Relative to normal[a]
Normal	68	0±0.2014	1 (0.87–1.15)	0±0.2512	1 (0.84–1.19)
Paratumor	68	−0.1551±0.1711	1.11 (0.98–1.25)	−0.0955±0.2437	1.07 (0.90–1.27)
Tumor	68	−2.1799±0.2509	4.53 (3.81–5.39)	−1.9387±0.3019	3.83 (3.11–4.73)

[a]Data are expressed as the mean $2^{-\Delta\Delta CT}$ (range).

Table 2 Correlation between *RhoA* and *RhoC* ΔΔCT values and clinical histopathological parameters.

Pathological features	Case number	ΔΔCT (*RhoA*)	p-Value	ΔΔCT (*RhoC*)	p-Value
Gender					
Male	38	−2.255±0.3621	>0.05	−1.9767±0.2611	>0.05
Female	30	−2.105±0.2766		−1.9007±0.3562	
Site of tumor					
Colon	31	−2.1675±0.2577	>0.05	−1.8614±0.2763	>0.05
Rectum	37	−2.1923±0.2132		−2.0160±0.3269	
Histological subtypes					
High and moderate differentiation	51	−2.0875±0.1734	<0.05	−1.9266±0.3326	>0.05
Low differentiation	17	−2.3602±0.2877		−1.9913±0.3125	
Extent of intestinal invasion					
T_1+T_2	24	−2.1405±0.2847	>0.05	−1.7005±0.2566	<0.05
T_3+T_4	44	−2.2036±0.1964		−2.0673±0.2137	
Lymph node metastasis					
Positive	24	−2.3503±0.1926	<0.05	−2.1503±0.1344	<0.05
Negative	44	−2.0732±0.2533		−1.8362±0.2581	
Liver metastasis					
Positive	11	−2.4106±0.0810	<0.05	−2.2662±0.1050	<0.05
Negative	55	−2.1422±0.2198		−1.9405±0.1911	

expression level correlates directly with the invasiveness of prostate cancer cells (26). In liver carcinoma, retroviral transduction of *RhoC* siRNA inhibits cellular motility (27).

Fluorescence-based QRT-PCR is a fast and accurate technique, and can be used for quantifying the levels of mRNA transcripts. It has been widely used in different fields of molecular biology. In this study, we characterized the relative levels of *RhoA* and *RhoC* mRNA transcripts in tissues by QRT-PCR. In theory, relative quantification requires comparison of the quantity of mRNA between samples derived from the same number of cells. This is very difficult in practice given the difficulties in obtaining the same number of cells from different samples. In addition, there is variability in RNA extraction and PCR amplification. We standardized the mRNA levels of our target genes against *GAPDH*, which is an internal housekeeping gene. Then we compared mRNA levels between different samples, improving the validity of the result (28, 29).

Colorectal carcinoma is a common malignancy. Local invasion and metastasis is a key factor compromising the efficacy of treatment. However, the underlying molecular and cellular mechanisms are poorly understood. In this study, we found that the levels of *RhoA* and *RhoC* mRNA transcripts in tumor tissue was significantly higher than that found in the corresponding paratumor and normal tissues. These results suggest involvement of these two genes in the onset and development of colorectal carcinoma. In addition, expression of both *RhoA* and *RhoC* in cancer with metastasis to lymph nodes or liver was significantly higher compared with cancer without metastasis. This indicates that these two genes may contribute to invasion and metastasis of colorectal carcinoma. Specifically, the levels of *RhoC* expression were significantly correlated with the extent of local invasion to intestine, but not with the histopathological degree of cancer. This strongly supports its function in tumor invasion and metastasis.

In gastric cancer, the adenovirus-mediated siRNA against *RhoA* and *RhoC* down-regulates the expression of both genes and inhibits the proliferation and invasiveness of cancer cells (30). Our study also identified positive correlation between mRNA levels of these two genes ($r=0.847$, $p<0.01$). This suggests cooperativity between these two genes in the initiation and progression of cancer, especially invasion and metastasis of colorectal carcinoma.

To our knowledge, this is the first study on the levels of *RhoA* and *RhoC* mRNA transcripts in tissue samples from Chinese patients with colorectal carcinoma by QRT-PCR; similar studies have been performed with other human tumors. Our results are consistent with a previous study done by Faried et al. that used essentially the same method in patients with squamous cell carcinoma of the esophagus (31). For quantitative methods, our TaqMan probes are more precise than SYBR Green chemistry dyes used by others. Another study used QRT-PCR and Western blotting on tissue from 64 patients with liver cancer

and found that the levels of *RhoA* mRNA transcripts were correlated with protein levels, and higher expression was correlated with tumor stage and metastasis (32). In ovarian cancer, levels of *RhoA* and *RhoC* mRNA and protein expression are up-regulated in cancer tissue, as determined by QRT-PCR and Western blot analysis (33). Shikada et al. examined mRNA and protein levels of *RhoC* in 49 non-small cell lung carcinoma. They found that mRNA levels were correlated with protein levels, with both significantly higher in tumor tissue, as compared with non-tumor tissue (34).

We believe that levels of *RhoA* and *RhoC* mRNA transcripts in colorectal carcinoma are correlated with protein expression. Further study on *RhoA* and *RhoC* expression in colorectal carcinoma may provide more insight into the mechanisms underlying the action of *RhoA* and *RhoC* in the development and metastasis of colorectal carcinoma. Therefore, *RhoA* and *RhoC* expression may be new markers of invasiveness of colorectal carcinoma, and used as potential targets for treatment.

Acknowledgements

This work was supported by grants from the Natural Scientific Foundation of Shandong Province (Grant code: 2006ZRB14274) and the Research Program of Qingdao South District Municipal Science and Technology Commission.

References

1. Jemal A, Ward E, Hao Y, Thun M. Trends in the leading causes of death in the United States, 1970–2002. J Am Med Assoc 2005;294:1255–9.
2. Nelson H, Petrelli N, Carlin A, Couture J, Fleshman J, Guillem J, et al. Guidelines 2000 for colon and rectal cancer surgery. J Natl Cancer Inst 2001;93:583–96.
3. Sahai E, Marshall CJ. RHO-GTPases and cancer. Nat Rev Cancer 2002;2:133–42.
4. Fritz G, Just I, Kaina B. Rho GTPases are over-expressed in human tumors. Int J Cancer 1999;81:682–7.
5. Esteve P, Embade N, Perona R, Jiménez B, del Peso L, León J, et al. Rho-regulated signals induce apoptosis in vitro and in vivo by a p53-independent, but Bcl2 dependent pathway. Oncogene 1998;17:1855–69.
6. Bellovin DI, Simpson KJ, Danilov T, Maynard E, Rimm DL, Oettgen P, et al. Reciprocal regulation of RhoA and RhoC characterizes the EMT and identifies RhoC as a prognostic marker of colon carcinoma. Oncogene 2006;25:6959–67.
7. Takami Y, Higashi M, Kumagai S, Kuo PC, Kawana H, Koda K, et al. The activity of RhoA is correlated with lymph node metastasis in human colorectal cancer. Dig Dis Sci 2008;53:467–73.
8. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 2001;25:402–8.
9. Yuan JS, Reed A, Chen F, Stewart CN Jr. Statistical analysis of real-time PCR data. BMC Bioinformatics 2006; 7:85.
10. Aspenstrom P, Fransson A, Saras J. Rho GTPases have diverse effects on the organization of the actin filament system. Biochem J 2004;377:327–37.
11. Leung T, Manser E, Tan L, Lim L. A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. J Biol Chem 1995;270:29051–4.
12. Matsui T, Amano M, Yamamoto T, Chihara K, Nakafuku M, Ito M, et al. Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho. EMBO J 1996;15:2208–16.
13. Lawler K, Foran E, O'Sullivan G, Long A, Kenny D. Mobility and invasiveness of metastatic esophageal cancer are potentiated by shear stress in a ROCK- and Ras-dependent manner. Am J Physiol Cell Physiol 2006;291: C668–77.
14. Imamura F, Mukai M, Ayaki M, Akedo H. Y-27632, an inhibitor of Rho-associated protein kinase, suppresses tumor cell invasion via regulation of focal adhesion and focal adhesion kinase. Jpn J Cancer Res 2000;91:811–6.
15. Somlyo AV, Bradshaw D, Ramos S, Murphy C, Myers CE, Somlyo AP. Rho-kinase inhibitor retards migration and in vivo dissemination of human prostate cancer cells. Biochem Biophys Res Commun 2000;269:652–9.
16. Benitah SA, Valeron PF, van Aelst L, Marshall CJ, Lacal JC. Rho GTPases in human cancer: an unresolved link to upstream and downstream transcriptional regulation. Biochem Biophys Acta 2004;1705:121–32.
17. Fiordalisi JJ, Keller PJ, Cox AD. PRL tyrosine phosphatases regulate rho family GTPases to promote invasion and motility. Cancer Res 2006;66:3153–61.
18. Kusama T, Mukai M, Iwasaki T, Tatsuta M, Matsumoto Y, Akedo H, et al. 3-hydroxy-3-methylglutaryl-coenzyme a reductase inhibitors reduce human pancreatic cancer cell invasion and metastasis. Gastroenterology 2002; 122:308–17.
19. Pille JY, Denoyelle C, Varet J, Bertrand JR, Soria J, Opolon P, et al. Anti-RhoA and anti-RhoC siRNAs inhibit the proliferation and invasiveness of MDA-MB-231 breast cancer cells in vitro and in vivo. Mol Ther 2005;11: 267–74.
20. Lin MT, Lin BR, Chang CC, Chu CY, Su HJ, Chen ST, et al. IL-6 induces AGS gastric cancer cell invasion via activation of the c-Src/RhoA/ROCK signaling pathway. Int J Cancer 2007;120:2600–8.
21. Sahai E, Olson MF, Marshall CJ. Cross-talk between Ras and Rho signalling pathways in transformation favours proliferation and increased motility. EMBO J 2001; 20:755–66.
22. Ikoma T, Takahashi T, Nagano S, Li YM, Ohon Y, Ando K, et al. A definitive role of RhoC in metastasis of orthotopic lung cancer in mice. Clin Cancer Res 2004;10: 1192–200.
23. Wang W, Yang LY, Huang GW, Lu WQ, Yang ZL, Yang JQ, et al. Genomic analysis reveals RhoC as a potential marker in hepatocellular carcinoma with poor prognosis. Br J Cancer 2004;90:2349–55.
24. Keely PJ. Rho GTPases as early markers for tumour progression. Lancet 2001;358:1744–5.
25. Clark EA, Golub TR, Lander ES, Hynes RO. Genomic analysis of metastasis reveals an essential role for RhoC. Nature 2000;406:532–5.
26. Yuan Z, Su J, You JF, Wang JL, Cui XL, Zheng J. Correlation of expression of RhoC with invasiveness of prostate cancer cell line PC-3M in vitro. Zhonghua Yi Xue Za Zhi 2008;88:51–5.
27. Tang NH, Wang XQ, Li XJ, Chen YL. Effect of retrovirus-mediated RhoC-siRNA on biological behaviors of human high-metastatic liver cancer cells. Chinese J Exp Surg 2006;23:1299–301.
28. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001; 29:e45.
29. Pfaffl MW, Horgan GW, Dempfle L. Relative expression

software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 2002;30:e36.
30. Sun HW, Tong SL, He J, Wang Q, Zou L, Ma SJ, et al. RhoA and RhoC-siRNA inhibit the proliferation and invasiveness activity of human gastric carcinoma by Rho/PI3K/Akt pathway. World J Gastroenterol 2007;13:3517–22.
31. Faried A, Faried LS, Usman N, Kato H, Kuwano H. Clinical and prognostic significance of RhoA and RhoC gene expression in esophageal squamous cell carcinoma. Ann Surg Oncol 2007;14:3593–601.
32. Wang D, Dou K, Xiang H, Song Z, Zhao Q, Chen Y, et al. Involvement of RhoA in progression of human hepatocellular carcinoma. J Gastroenterol Hepatol 2007;22:1916–20.
33. Horiuchi A, Imai T, Wang C, Ohira S, Feng Y, Nikaido T, et al. Up-regulation of small GTPases, RhoA and RhoC, is associated with tumor progression in ovarian carcinoma. Lab Invest 2003;83:861–70.
34. Shikada Y, Yoshino I, Okamoto T, Fukuyama S, Kameyama T, Maehara Y. Higher expression of RhoC is related to invasiveness in non-small cell lung carcinoma. Clin Cancer Res 2003;9:5282–6.