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World J Gastroenterol 2005;11(32):5006-5009
World Journal of Gastroenterology ISSN 1007-9327

• *BRIEF REPORTS* •

Pin1 overexpression in colorectal cancer and its correlation with aberrant β-catenin expression

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Supported by the Korea Science and Engineering Foundation (KOSEF) through the Cell Death Disease Research Center at The Catholic University of Korea, No. R13-2002-005-01004-0
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Received: 2004-11-23 Accepted: 2005-01-05

Abstract

AIM: To investigate clinical significance of Pin1 and β-catenin expression in colorectal cancers and to demonstrate the relationship of their expression.

METHODS: The role of Pin1 and β-catenin protein in colorectal tumorigenesis and their clinicopathologic significance were analyzed by immunohistochemistry, and the correlation between Pin1 and β-catenin protein expressions was also studied in 124 patients with colorectal cancer who were surgically treated.

RESULTS: Normal colonic epithelium either failed to express or showed focal and weak expression of Pin1 and β-catenin. Overexpression of Pin1 and β-catenin protein was found in 23 (18.54%) and 50 (40.3%) of 124 colorectal cancers, respectively. Overexpression of both proteins was not related to the lymph node metastasis, tumor stage and survival period after excision. Survival analysis results indicated that tumor stage was a valuable predictor of survival. Interestingly, a significant correlation was found between Pin1 and β-catenin protein expression.

CONCLUSION: Overexpression of Pin1 and β-catenin may be closely related with the development and/or progression of colorectal carcinoma and further supports that Pin1 overexpression might contribute to the upregulation of β-catenin.

Key words: Pin1; Immunohistochemistry; β-catenin; Survival

Kim CJ, Cho YG, Park YG, Nam SW, Kim SY, Lee SH, Yoo NJ, Lee JY, Park WS. Pin1 overexpression in colorectal cancer and its correlation with aberrant β-catenin expression. *World J Gastroenterol* 2005; 11(32): 5006-5009
http://www.wjgnet.com/1007-9327/11/5006.asp

INTRODUCTION

β-catenin is a multifunctional protein that plays an important role in the transduction of Wnt signals and in the intercellular adhesion by linking the cytoplasmic domain of cadherin[1]. In general, the cytoplasmic level of β-catenin is kept low through interaction with a protein complex, comprised of adenomatous polyposis coli (APC), Axin, protein phosphatase 2A, and glycogene synthase kinase 3β (GSK3β). It is believed that this complex phosphorylates the β-catenin, thereby inducing ubiquitination-dependent proteolysis of β-catenin. Therefore, alterations of these genes cause accumulation of cytoplasmic β-catenin and nuclear translocation of β-catenin. After its translocation into the nucleus, β-catenin binds to members of the Tcf/Lef family thereby activating target genes, such as cyclin D1 and myc. In cancer cells, only one of these genes is mutated in a given tumor sample reflecting their role in a common pathway[2]. For instance, colon tumor with mutations in APC has α wild-type β-catenin gene, and vice versa, any tumor with mutations in β-catenin is wild-type for APC.

Recently, it has been shown that Pin1 is overexpressed in some human malignancies and that its expression closely correlates with the level of cyclin D1 in human cancer[3]. Pin1 is a peptidyl-prolyl cis-trans isomerase that isomerizes only phosphorylated serine/threonine residues preceding proline peptide bonds to regulate various cellular processes including cell division and transcription[4-7]. Interestingly, Pin1 contributes to the upregulation of β-catenin in tumors such as breast cancer by inhibiting interaction between APC and β-catenin[8]. Thus, this study aimed to elucidate, whether Pin1 and β-catenin expressions were involved in colorectal carcinogenesis and whether Pin1 expression contributed to aberrant β-catenin overexpression.

MATERIALS AND METHODS

Patients and specimens

One hundred and twenty-four patients with colorectal cancers from January 2001 to December 2002, were enrolled in this study. No patient had a family history of colorectal cancer and was treated with chemotherapy before tumor removal.

Tumor stage was classified according to Dukes' criteria. Thirteen patients were classified as Dukes' A, 47 as Dukes' B, 56 as Dukes' C and 8 as Dukes' D. The range of observation was 14-36 mo for the survivors. Of these, 15 patients showed relapse of cancer and 11 patients died of cancer during this time. Specimens collected from these patients were fixed by formalin and embedded in paraffin. Two pathologists screened histological sections and selected areas of the representative tumor cells. Three tissue cores (0.6 mm in diameter) were taken from each tumor sample and placed in a new recipient paraffin block using a commercially available microarray instrument (Beecher Instruments, Micro-Array Technologies, Silver Spring, MD, USA) according to established methods[9]. One cylinder of normal colonic mucosa adjacent to each tumor was also transferred to the recipient block.

Immunohistochemistry for Pin1 and β-catenin

Primary polyclonal rabbit anti-Pin1 antibody (Oncogene Research Products, San Diego, CA, USA, dilution 1/100) and anti-β-catenin (Transduction Laboratories, Lexington, KY, USA) were used. Immunostaining was performed on microarray tissue sections with a tyramide signal amplification kit (NEN Life Science, Boston, MA, USA) for signal intensification. Antigen retrieval was performed by microwave heating in a citrate buffer (pH 6.0). Other procedures were performed as previously described[10]. The reaction products were developed with diaminobenzidine (Sigma, St. Louis, MO, USA) and counterstained with hematoxylin. A negative control, using non-immune rabbit serum instead of the primary antisera, did not produce any staining (data not shown). Three pathologists independently reviewed the results. Immunoreactivities of both Pin1 and β-catenin were categorized into four groups: (1) negative, 0-5%; (2) low, 5-30%; (3) moderate, 30-50%; (4) high, ⩾50%. Since both Pin1 and β-catenin expressions were negative or low in normal colonic mucosa, we considered moderate and high immunoreactivities as overexpression.

Statistical analysis

We used χ^2 test to analyze the correlation between clinicopathologic parameters of colorectal cancer and expressions of Pin1 and β-catenin, and the association between Pin1 and β-catenin expressions. $P<0.05$ was considered statistically significant. The predictive value of clinical parameters for survival was evaluated using the Kaplan-Meier analysis.

RESULTS

Pin1 protein expression in colorectal cancer

One hundred and twenty-four colorectal carcinomas were screened for Pin1 protein expression. The expression was mainly negative or low in normal colonic mucosa. In the present study, overexpression of Pin1 was found in 23 (18.5%) of the 124 colorectal carcinomas, in which immunostaining was predominant in either the cytoplasm or the nuclei of tumor cells (Figure 1). Of these 23 colorectal carcinomas, 3 had high expression of Pin1 and 20 had moderate expression of Pin1. Positive staining was seen in 23.1% (3 of 13 cases) of stage A patients, 23.4% (11 of 47) of stage B patients, 12.5% (7 of 56) of stage C patients, and 25.0% (2 of 8) of stage D patients, respectively (Table 1). There was no significant correlation between overexpression of Pin1 and Dukes' stage. In addition, Pin1 expression was detected in 8 (13.6%) of 59 cases with lymph node metastasis and showed no significant correlation with lymph node metastasis. Univariate analysis showed that the expression of Pin1 in colorectal cancer was not related with survival period after excision, indicating that the presence of Pin1 staining was not a valuable predictor of survival.

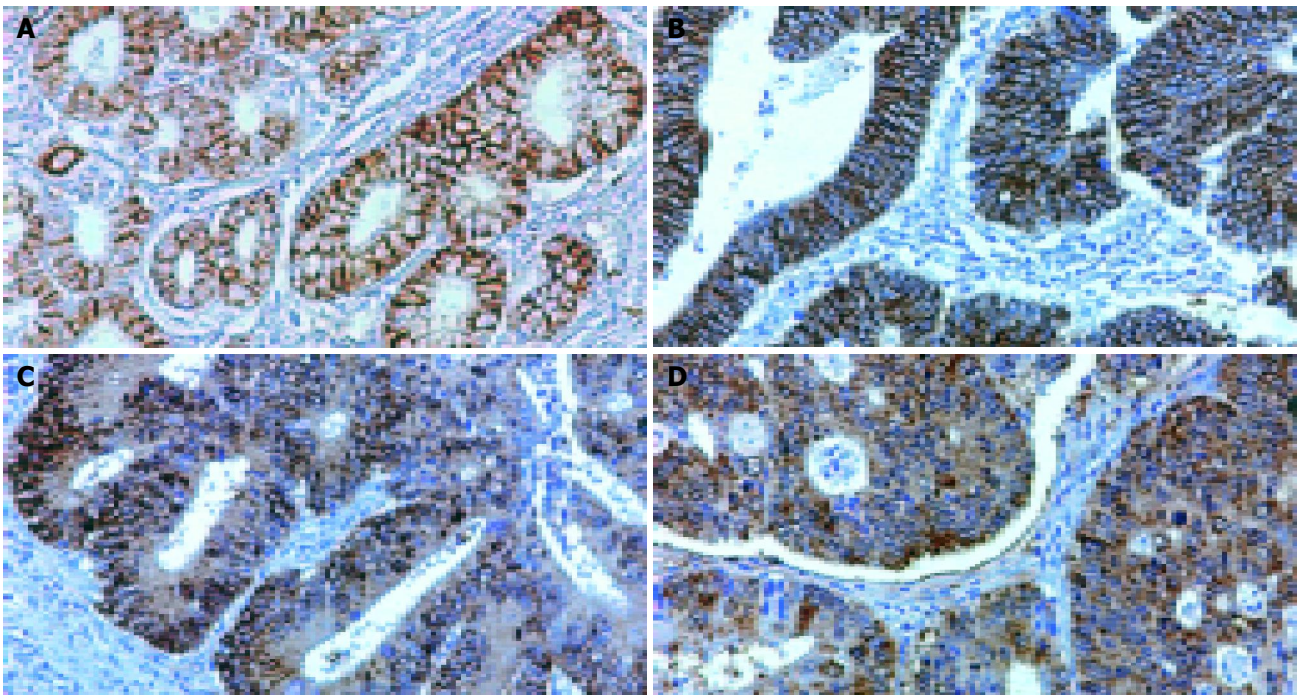

Figure 1 Expression of β-catenin and Pin1 proteins in normal colonic mucosa (**A**) and cancer cells (**C**-**D**) (**B** and **C**, β-catenin; **D**, Pin1).

Table 1 Relationship between expression of Pin1 and β-catenin protein and clinicopathologic parameters

Groups	Pin1		Positive (%)	*P*	β-catenin		Positive (%)	*P*
	+	-			+	-		
Stage				0.4778				0.6774
A	3	10	23.1		6	7	46.2	
B	11	36	23.4		21	26	44.7	
C	7	49	12.5		21	35	37.5	
D	2	6	25.0		2	6	25.0	
L/N metastasis				0.2474				0.5114
+	8	51	13.6		22	37	37.3	
-	15	50	23.1		28	37	43.1	
Survival period				0.3312				0.5154
< 24 mo	3	7	30.0		5	5	50.0	
⩾24 mo	20	94	17.5		45	69	15.8	
Pin1 protein								0.000004
+					19	4	82.6	
-					31	70	30.7	
Total	23	101	18.5		50	74	40.3	

L/N: lymph node.

Expression of β-catenin protein

Expression of β-catenin protein was found on cell membrane in the normal colonic mucosa. The cancer cells demonstrated abnormal nuclear and cytoplasmic staining of β-catenin, whereas the membrane staining was negative or low in tumors (Figure 1). Overexpression was observed in 50 (40.3%) of 124 specimens. There was no significant correlation between β-catenin expression and clinicopathologic parameters, including lymph node metastasis, tumor stage and survival period after surgical resection (Table 1). Overall, only tumor stage was a significant predictor of survival, and lower stage patients had a longer survival as shown in log rank test ($P<0.05$).

Correlation between Pin1 and β-catenin

Overexpression of Pin1 protein was detected in 19 of 50 colorectal cancers with aberrant expression of β-catenin protein. There was a significant positive correlation between the expressions of Pin1 and β-catenin ($P<0.01$, Table 1).

DISCUSSION

Phosphorylation of proteins on serine/threonine residues preceding proline is a major regulatory mechanism in cell proliferation and transformation[11,12]. The prolyl isomerase Pin1, catalyzes conformational changes in certain key proline-directed phosphorylation sites and functions as a pivotal catalyst for oncogenesis[13]. Pin1 is overexpressed in many human tumors such as breast and prostate cancer[14-16], and increases the transcriptional activity of c-Jun towards the cyclin D1 promoter[3]. In the present study, we found that Pin1 protein levels in colorectal cancer cells were not positively correlated with any commonly used clinicopathologic parameters, such as tumor stage and lymph node metastasis. Additionally, univariate analysis demonstrated that there was no association between Pin1 expression and survival, suggesting that the Pin1 level might not a valuable prognostic marker for predicting the overall survival of colorectal cancer patients.

β-catenin is a multifunctional protein, and plays an essential role in the transduction of Wnt signals. Cytosolic β-catenin is eliminated by APC-dependent proteasomal degradation pathways regulated by GSK3β or p53-inducible Siah-1. Conditional degradation of β-catenin represents a central event of Wnt signaling pathway controlling cell fate and proliferation[2]. Dysregulation of β-catenin turnover caused by genetic alterations of Wnt signaling pathway-related genes is implicated in cancers[17]. Alterations of β-catenin expression have been documented in many malignancies, including breast cancer, gastric cancer, colonic and hepatocellular carcinomas[18-21]. Additionally, overexpression of Pin1 can upregulate β-catenin in tumors, by inhibiting interaction between APC and β-catenin[8]. We also found that β-catenin was abnormally expressed in 50 (40.3%) of 124 colorectal cancers. The expression was not related to clinical parameters and survival period after surgical excision. By comparing survival and clinicopathologic features, we found that only tumor stage was positively correlated with survival in log rank test ($P<0.05$).

Interestingly, statistical analysis revealed a significant correlation between Pin1 overexpression and β-catenin expression, suggesting that Pin1 overexpression is significantly correlated with β-catenin expression in colorectal cancer and further supports, that aberrant Pin1 expression may be important for the upregulation of β-catenin expression.

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Science Editor Wang XL and Guo SY **Language Editor** Elsevier HK