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

Chang-Jae Kim, Yong-Gu Cho, Yong-Gyu Park, Suk-Woo Nam, Su-Young Kim, Sung-Hyung Lee, Nam-Jin Yoo, Jung-Young Lee, Won-Sang Park

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 a 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]. For instance, Pin1 contributes to the upregulation of β-catenin in tumors such as breast cancer by inhibiting interaction between APC and β-catenin[8]. Pin1 overexpression might contribute to the upregulation of β-catenin.

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.

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Keywords: Pin1; Immunohistochemistry; β-catenin; Survival

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.

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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.
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 \(\chi^2\) 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).
Expression of \( \beta \)-catenin protein

Expression of \( \beta \)-catenin protein was found on cell membrane in the normal colonic mucosa. The cancer cells demonstrated abnormal nuclear and cytoplasmic staining of \( \beta \)-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 \( \beta \)-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 (Figure 1).

Correlation between Pin1 and \( \beta \)-catenin

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

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[14,15], and increases the transcriptional activity of c-Jun towards the cyclin D1 promoter[16]. 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.

\( \beta \)-catenin is a multifunctional protein, and plays an essential role in the transduction of Wnt signals. Cytosolic \( \beta \)-catenin is eliminated by APC-dependent proteasomal degradation pathways regulated by GSK3\( \beta \) or p53-inducible Siah-1. Conditional degradation of \( \beta \)-catenin represents a central event of Wnt signaling pathway controlling cell fate and proliferation[17]. Dysregulation of \( \beta \)-catenin turnover caused by genetic alterations of Wnt signaling pathway-related genes is implicated in cancers[18]. Alterations of \( \beta \)-catenin expression have been documented in many malignancies, including breast cancer, gastric cancer, colonic and hepatocellular carcinomas[19-21]. Additionally, overexpression of Pin1 can upregulate \( \beta \)-catenin in tumors, by inhibiting interaction between APC and \( \beta \)-catenin[8]. We also found that \( \beta \)-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 \( \beta \)-catenin expression, suggesting that Pin1 overexpression is significantly correlated with \( \beta \)-catenin expression in colorectal cancer and further supports, that aberrant Pin1 expression may be important for the upregulation of \( \beta \)-catenin expression.

REFERENCES

1. Peifer M, Polakis P. Wnt signaling in oncogenesis and embryogenesis-a look outside the nucleus. Science 2000; 287: 1606-1609
2. Lustig B, Behrens J. The Wnt signaling pathway and its role in tumor development. J Cancer Res Clin Oncol 2003; 129: 199-221
3. Wulf GM, Ryo A, Wulf GG, Lee SW, Niu T, Petkova V, Lu KP. Pin1 is overexpressed in breast cancer and cooperates with Ras signaling in increasing the transcriptional activity of c-Jun towards cyclin D1. EMBO J 2001; 20: 3459-3472
4. Hunter T, Karin M. The regulation of transcription by phosphorylation. Cell 1992; 70: 375-387
5. Lu KP, Hanes SD, Hunter T. A human peptidyl-prolyl isomerase essential for regulation of mitosis. Nature 1996; 380: 544-547
6. Ranganathan R, Lu KP, Hunter T, Noel JP. Structural and
functional analysis of the mitotic rotamase Pin1 suggests that substrate recognition is phosphorylation dependent. Cell 1997; 89: 875-886

7 Shen M, Stukenberg PT, Kirschner MW, Lu KP. The essential mitotic peptidyl-prolyl isomerase Pin1 binds and regulates mitosis-specific phosphoproteins. Genes Dev 1998; 12: 706-720

8 Ryo A, Nakamura M, Wulf G, Liou YC, Lu KP. Pin1 regulates turnover and subcellular localization of β-catenin by inhibiting its interaction with APC. Nat Cell Biol 2001; 3: 793-801

9 Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4: 844-847

10 Park WS, Oh RR, Kim YS, Park JY, Shin MS, Lee HK, Lee SH, Yoo NJ, Lee JY. Absence of mutations in the kinase domain of the Met gene and frequent expression of Met and HGF/SF protein in primary gastric carcinomas. APMIS 2000; 108: 195-200

11 Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature 2001; 411: 355-365

12 Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70

13 Lu KP. Prolyl isomerase Pin1 as a molecular target for cancer diagnostics and therapeutics. Cancer Cell 2003; 4: 175-180

14 Nakashima M, Meirmanov S, Naruke Y, Kondo H, Saenko V, Rogoumovitch T, Shimizu-Yoshida Y, Takamura N, Namba H, Ito M, Abrosimov A, Lushnikov E, Roumiantsiev P, Tsyb A, Yamashita S, Sekine I. Cyclin D1 overexpression is thyroid tumours from a radio-contaminated area and its correlation with Pin1 and aberrant β-catenin expression. J Pathol 2004; 202: 446-455

15 Bao L, Kimzey A, Sauter G, Sowadski JM, Lu KP, Wang DG. Prevalent overexpression of prolyl isomerase Pin1 in human cancers. Am J Pathol 2004; 164: 1727-1737

16 Ayala G, Wang D, Wulf G, Frolow A, Li R, Sowadski J, Wheeler TM, Lu KP, Bao L. The prolyl isomerase Pin1 is a novel prognostic marker in human prostate cancer. Cancer Res 2003; 63: 6244-6251

17 Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996; 87: 159-170

18 Bukholm IK, Nesland JM, Borresen-Dale AL. Re-expression of E-cadherin, alpha-catenin and beta-catenin, but not of gamma-catenin, in metastatic tissue from breast cancer patients. J Pathol 2000; 190: 15-19

19 Woo DK, Kim HS, Lee HS, Kang YH, Yang HK, Kim WH. Altered expression and mutation of beta-catenin gene in gastric carcinomas and cell lines. Int J Cancer 2001; 95: 108-113

20 Utsunomiya T, Doki Y, Takemoto H, Shiozaki H, Yano M, Sekimoto M, Tamura S, Yasuda T, Fujiwara Y, Monden M. Correlation of beta-catenin and cyclin D1 expression in colon cancers. Oncology 2001; 61: 226-233

21 Pang R, Yuen J, Yuen MF, Lai CL, Lee TK, Man K, Poon RT, Fan ST, Wong CM, Ng IO, Kwong YL, Tse E. PIN1 overexpression and β-catenin gene mutations are distinct oncogenic events in human hepatocellular carcinoma. Oncogene 2004; 23: 4182-4186