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Expression of Cyclin D1 Is Associated with β-Catenin Expression and Correlates with Good Prognosis in Colorectal Adenocarcinoma[1]

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Abstract

BACKGROUND: The aim of this study is to investigate the prevalence and prognostic impact of β-catenin and cyclin D1 expression in colorectal carcinoma (CRC) patients. *METHOD:* We evaluated immunohistochemial expression of β-catenin and cyclin D1 using 2-mm cores from 220 CRC patients for tissue microarray, and its significance was statistically evaluated. *RESULT:* Positive expression of β-catenin and cyclin D1 was found in 72.5% (158 of 218 cases) and 59.4% (129 of 217 cases) of CRC patients, respectively. Expression of β-catenin was significantly correlated with tumor location ($P = .017$), differentiation ($P = .010$), lymph node metastasis ($P = .032$), preoperative carcinoembryonic antigen level ($P = .032$), and cyclin D1 expression ($P = .005$). Expression of cyclin D1 was significantly correlated with recurrence and/or metastasis ($P = .004$). In univariate analysis, β-catenin expression predicted more favorable overall survival ($P = .022$) and cyclin D1 expression predicted both more favorable overall survival and relapse-free survival ($P = .004$ and $P = .006$, respectively). Multivariate analysis showed that tumor stage and expression of cyclin D1 were independent prognostic factors significantly associated with overall survival and relapse-free survival. *CONCLUSION:* This study shows that expression of β-catenin and cyclin D1 is associated with favorable clinicopathologic variables and it is a clinically significant prognostic indicator for CRC patients.

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Introduction

Colorectal carcinoma (CRC) is the third most common malignant tumor worldwide, with an incidence of 1,230,000 cases and 610,000 deaths annually, making it the fourth most common cause of cancer deaths throughout the world [1]. Recently, there were considerable advances in diagnosis and treatment of CRCs. However, CRC is still associated with a high rate of incidence and mortality. The 5-year survival for CRC is less than 60% in Europe, and about one third of patients with CRC die from it [2].

The most commonly mutated gene in all CRCs is the *adenomatous polyposis coli* (*APC*) tumor suppressor gene that produces the APC protein [3]. Because the APC protein is a controller on the accumulation of β-catenin protein, loss of functional APC protein results in the accumulation of β-catenin [4]. β-Catenin is a member of the cadherin-catenin complex that mediates homotypic cell-cell adhesion [5]. Accumulated β-catenin is translocated into the nucleus, binds to DNA, and activates genes that are responsive to transcription factors of the T-cell factor/lymphoid enhancer factor family [6,7], resulting in the transcription of target genes such as cyclin D1 and matrix metalloproteinase [8,9]. Clinical significance of β-catenin expression in CRC was controversial. In some studies, expression of β-catenin was regarded as a factor of unfavorable prognosis in CRC [10,11], whereas other studies reported that it had no significant association with clinicopathologic factors [12].

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Cyclin D1 belongs to the highly conserved cyclin family and plays a key role in cell cycle control, particularly in the transition from G_1 to S phase [13]. Cyclin D1 can carry out multiple functions as an oncogene, by enhancing several processes during malignant cell transformation, including abnormal growth, angiogenesis, and resistance to apoptosis [14]. In addition, cyclin D1 is a target gene of the WNT/β-catenin pathway. It has been suggested that cyclin D1 transactivation secondary to APC or β-catenin mutations participates in colonic cancer initiation [8,15]. Overexpression of cyclin D1 occurs in one third or more of CRCs [16–22]. Though the role of cyclin D1 in cell cycle progression is relatively well established, there are some controversies on the relation of cyclin D1 expression and clinical outcome in CRCs. Some studies have demonstrated that cyclin D1 expression was associated with poor prognosis [16,17]; however, other studies have shown good prognosis associated with cyclin D1 expression [18,19]. The other studies demonstrated no prognostic significance of cyclin D1 expression [20–22].

Many studies have investigated the clinicopathologic and prognostic significance of β-catenin as well as cyclin D1 in CRCs; however, the results were quite different according to the studies and there was no investigation on the expression between β-catenin and cyclin D1 in CRCs. The aim of the present study is to determine the expression of β-catenin and cyclin D1 in CRC patients and to evaluate their correlation with the clinicopathologic variables and their prognostic impact on CRC patients.

Materials and Methods

Patients and Specimens

Two hundred twenty cases of colorectal adenocarcinoma patients who had surgical resection in Chonbuk National University Hospital between January 2003 and December 2004 were included in the present study. All of 220 patients had received complete surgical resection of the tumor with microscopically clear resection margins. Histologic findings of all cases were reviewed by two authors (Y.N.K. and H.S.P.) who were blinded to each other's evaluation. Among the 220 patients, 111 patients had received surgical resection only and 109 patients had received surgical resection and postoperative adjuvant chemotherapy (78 patients received 5-fluorouracil (5-FU), 14 patients received 5-FU plus folic acid and oxaliplatin, 6 patients received irinotecan, 5 patients received 5-FU plus folic acid, 4 patients received enteric-coated granular chemotherapeutic drug composed of tegafur and uracil (UFT-E), and 2 patients received oxaliplatin). The tissue microarray blocks were produced by using formalin-fixed paraffin-embedded tissue blocks. Two cores with a diameter of 2.0 mm were taken from colorectal adenocarcinoma in each cases. In addition, 20 cases of noncancerous colorectal mucosa were also included. The patients were grouped according to their age, sex, location, tumor size, differentiation, lymphovascular invasion, presence of lymph node metastasis, presence of recurrence and/or distant metastasis, stage (I and II *versus* III and IV), preoperative serum carcinoembryonic antigen (CEA) levels, and preoperative serum CA19-9 levels. The present study was approved by the local ethics committee of the institutional review board of Chonbuk National University Hospital.

Immunohistochemical Staining and Scoring

Immunohistochemistry was done by using the tissue microarray block. Briefly, after deparaffinization, tissue sections were treated with a microwave antigen retrieval procedure in 0.01 M sodium citrate buffer for 12 minutes. After blocking endogenous peroxidase, sections were incubated with Protein Block Serum-Free (Dako, Carpinteria, CA) at room temperature for 10 minutes to block nonspecific staining; the sections were then incubated for 2 hours at room temperature with anti–β-catenin (1:100, clone 14/β-catenin; BD Bioscience, San Jose, CA) and anti–cyclin D1 (1:50, clone SP4; Thermo Fisher Scientific Inc, Kalamazoo, MI) antibodies. Peroxidase activity was detected with the enzyme substrate 3-amino-9-ethyl carbazole. For the negative controls, sections were treated in the same manner, except that they were incubated with TBS without the primary antibody. Immunohistochemical analysis was done by three authors (K.Y.J., Y.N.K., and H.S.P.) without knowledge of the clinicopathologic information. Three authors simultaneously evaluated the slides for immunohistochemical stain by using the multiviewing microscope. In every case, the consensus for immunohistochemical score was reached after discussion on the intensity and area of immunostain by three authors. During the immunostaining, there were unexpected loss of tissue cores in two cases for β-catenin and three cases for cyclin D1. Accordingly, 218 cases of CRCs were immunostained for β-catenin and 217 cases of CRCs for cyclin D1. Each case was evaluated by estimating the percentages of tumor cells that stained positively for each marker. Immunostaining for β-catenin and cyclin D1 was considered positive if $\geq$30% of the tumor cells in either core were stained with an antibody. Paraffin-embedded tissue samples for immunohistochemistry were provided by the Chonbuk National University Hospital, a member of the National Biobank of Korea, which is supported by the Ministry of Health, Welfare and Family Affairs.

Statistical Analysis

The end points of interest were relapse-free survival and overall survival. The end point of follow-up was the date of the last contact or the date of death through November 2011. Overall survival was calculated as the time from diagnosis to the date of death or last contact. Patients who were alive at last contact were treated as censored for overall survival analysis. Relapse-free survival was calculated from the time of diagnosis to the date of recurrence, metastasis, death, or last contact. Patients who were alive at last contact and who had not recurred and not metastasized were treated as censored for relapse-free survival analysis. The associations between staining index and other categorical factors potentially predictive of prognosis were analyzed using Pearson χ^2 test. Univariate and multivariate Cox proportional hazards regression analysis was done to estimate the impact of clinicopathologic factors and expression of each marker on relapse-free survival and overall survival. Kaplan-Meier survival curves were constructed to further illustrate the impact of overall survival when indicated. SPSS software (version 18.0; SPSS Inc, Chicago, IL) was used throughout for statistical analysis, and $P < .05$ was considered statistically significant.

Results

Association of β-Catenin and Cyclin D1 Expression with Clinicopathologic Characteristics of CRCs

The clinicopathologic features are summarized in Table 1. Immunohistochemical staining of β-catenin and cyclin D1 in normal colorectal mucosa and colorectal adenocarcinoma tissues are shown in Figure 1. Expression of β-catenin and cyclin D1 was not identified in all 20 cases of normal colorectal mucosa. In colorectal adenocarcinoma, immunoreactivity for cyclin D1 was found primarily in the nuclei. Although

Table 1. Clinicopathologic Variables and the Expression Status of β-Catenin and Cyclin D1.

	β-Catenin				Cyclin D1			
	No. of Cases	Negative (%)	Positive (%)	*P* Value	No. of Cases	Negative (%)	Positive (%)	*P* Value
Age (years)				0.290				0.074
≤65	118	29 (24.6)	89 (75.4)		117	41 (35.0)	76 (65.0)	
>65	100	31 (31.0)	69 (69.0)		100	47 (47.0)	53 (53.0)	
Gender				0.369				0.791
Male	134	34 (25.4)	100 (74.6)		133	53 (39.8)	80 (60.2)	
Female	84	26 (31.0)	58 (69.0)		84	35 (41.7)	49 (58.3)	
Location				0.017				0.327
Rt. colon	51	22 (43.1)	29 (56.9)		50	16 (32.0)	34 (68.0)	
Lt. colon	64	14 (21.9)	50 (78.1)		64	26 (40.6)	38 (59.4)	
Rectum	103	24 (23.3)	79 (76.7)		103	46 (44.7)	57 (55.3)	
Tumor size (cm)				0.623				0.942
≤4.0	82	21 (25.6)	61 (74.4)		82	33 (40.2)	49 (59.8)	
>4.0	136	39 (28.7)	97 (71.3)		135	55 (40.7)	80 (59.3)	
Differentiation				0.010				0.526
Well/moderate	60	50 (83.3)	10 (16.7)		198	79 (39.9)	119 (60.1)	
Poor	158	149 (94.3)	9 (5.7)		19	9 (47.4)	10 (52.6)	
Lymphovascular invasion				0.166				0.609
Absent	170	43 (25.3)	127 (74.7)		169	67 (39.6)	102 (60.4)	
Present	48	17 (35.4)	31 (64.6)		48	21 (43.8)	27 (56.3)	
Lymph node metastasis				0.032				0.188
Absent	123	27 (22.0)	96 (78.0)		123	45 (36.6)	78 (63.4)	
Present	91	32 (35.2)	59 (64.8)		90	41 (45.6)	49 (54.4)	
Recurrence/metastasis				0.564				0.004
No	200	54 (27.0)	146 (73.0)		199	75 (37.7)	124 (62.3)	
Yes	18	6 (33.3)	12 (66.7)		18	13 (72.2)	5 (27.8)	
Stage				0.066				0.308
I and II	120	27 (22.5)	93 (77.5)		120	45 (37.5)	75 (62.5)	
III and IV	98	33 (33.7)	65 (66.3)		97	43 (44.3)	54 (55.7)	
Preoperative CEA (ng/ml)				0.032				0.782
≤5.0	141	32 (22.7)	109 (77.3)		141	54 (38.3)	87 (61.7)	
>5.0	68	25 (36.8)	43 (63.2)		67	27 (40.3)	40 (59.7)	
Preoperative CA19-9 (U/ml)				0.380				0.390
≤37.0	136	39 (28.7)	97 (71.3)		135	51 (37.8)	84 (62.2)	
>37.0	21	8 (38.1)	13 (61.9)		21	10 (47.6)	11 (52.4)	
Cyclin D1				0.005				
Negative	59	33 (55.9)	26 (44.1)					
Positive	158	55 (34.8)	103 (65.2)					

β-catenin was expressed in the nuclei, membrane, and cytoplasm, we evaluated nuclear β-catenin expression only. As shown in Table 1, positive expression of β-catenin was found in 72.5% (158 of 218 cases) and of cyclin D1 in 59.4% (129 of 217 cases) of colorectal adenocarcinoma patients. Expression of β-catenin was significantly correlated with tumor location ($P = .017$), differentiation ($P = .010$), lymph node metastasis ($P = .032$), preoperative CEA level ($P = .032$), and cyclin D1 expression ($P = .005$). Stage showed a tendency to be correlated with β-catenin expression ($P = .066$). There was no correlation between β-catenin expression and age, sex, tumor size, lymphovascular invasion, recurrence and/or metastasis, and preoperative CA19-9 level. Expression of cyclin D1 was also significantly correlated with recurrence and/or metastasis ($P = .004$). There was no correlation between cyclin D1 expression and age, sex, location, tumor size, lymphovascular invasion, lymph node metastasis, stage, preoperative CEA level, and preoperative CA19-9 level.

Expression of β-Catenin and Cyclin D1 in Colorectal Adenocarcinoma Correlates with Favorable Relapse-free Survival and Overall Survival

Univariate Cox proportional hazard analysis of the expression of β-catenin and cyclin D1 and their relationship with relapse-free survival and overall survival are shown in Table 2. Tumor size larger than 4.0 cm ($P = .011$ and $P = .004$), poorly differentiated tumor ($P < .001$ and $P = .006$, Figure 2*A*), presence of lymph node metastasis ($P < .001$ and $P = .002$), advanced stage (III and IV, $P < .001$ and $P < .001$, Figure 2*B*), preoperative CEA level more than 5.0 ng/ml ($P = .002$ and $P < .001$), and preoperative CA19-9 level more than 37.0 U/ml ($P < .001$ and $P < .001$) predicted both shorter overall survival and relapse-free survival, respectively. In addition, age more than 65 years ($P = .009$) predicted shorter overall survival and presence of lymphovascular invasion ($P = .008$) predicted shorter relapse-free survival. However, expression of β-catenin ($P = .022$, Figure 2*C*) also predicted more favorable overall survival and expression of cyclin D1 ($P = .004$ and $P = .006$, Figure 2*D*) predicted both more favorable overall survival and relapse-free survival, respectively. There was no prognostic significance between the patients treated with surgery only and the patients who received adjuvant chemotherapy after surgical resection (overall survival, $P = .625$; relapse-free survival, $P = .482$; Table 2). When we separately considered the patients according to the treatment modality, the expression of β-catenin predicted more favorable overall survival in patients who received adjuvant chemotherapy ($P = .018$; Figure 3*A*). Cyclin D1 expression was also significantly correlated with better overall survival ($P < .001$) and relapse-free survival ($P = .03$) in the patients who received adjuvant chemotherapy (Figure 3*B*). However, the expression of β-catenin or cyclin D1 was not associated with overall survival (β-catenin, $P = .422$; cyclin D1, $P = .867$) or relapse-free survival (β-catenin, $P = .539$; cyclin D1, $P = .069$) in the patients treated with surgical resection only.

Expression of Cyclin D1 in Colorectal Adenocarcinoma Is an Independent Prognostic Factor for Good Survival Outcome

Multivariate analysis was performed using 156 patients with complete information for all variables. Variables considered in the analysis were the age, sex, location, tumor size, differentiation, lymphovascular invasion, stage, preoperative CEA level, preoperative CA19-9 level, β-catenin expression, and cyclin D1 expression. From the multivariate analysis, only stage and expression of cyclin D1 were independent prognostic factors significantly associated with both overall survival and relapse-free survival (Table 3). Advanced stage (III and IV) was an independent prognostic factor significantly associated with poor overall survival (adjusted hazard ratio (HR), 2.540; 95% confidence interval (CI), 1.388–4.649; $P = .003$) and relapse-free survival (adjusted HR, 2.857; 95% CI, 1.421–5.745; $P = .003$). Colorectal adenocarcinomas with cyclin D1 expression were associated with a significantly higher overall survival (adjusted HR, 0.477; 95% CI, 0.279–0.817; $P = .007$) and relapse-free survival (adjusted HR, 0.508; 95% CI, 0.282–0.916, $P = .024$). In addition, age more than 65 years, poorly differentiated tumor, and preoperative CA19-9 level more than 37.0 U/ml were also independent prognostic factors significantly associated with overall survival. Among the patients who received adjuvant chemotherapy, expression of β-catenin was an independent prognostic factor significantly associated with good overall survival (adjusted HR, 0.406; 95% CI, 0.194–0.850, $P = .017$). Moreover, expression of cyclin D1 in patients who received adjuvant chemotherapy was also an independent prognostic factor significantly associated with good overall survival (adjusted HR, 0.025; 95% CI, 0.092–0.457, $P < .001$) and relapse-free survival (adjusted HR, 0.452; 95% CI, 0.212–0.963, $P < .040$).

Discussion

In this study, we examined the immunohistochemical expression of β-catenin and cyclin D1 in colorectal adenocarcinoma tissues, their correlation with clinicopathologic variables, and their prognostic significance. This study has shown that 1) 72.5% of colorectal adenocarcinomas expressed β-catenin and its expression was significantly correlated with favorable clinicopathologic variables such as well or moderate differentiation, absence of lymph node metastasis, low preoperative serum CEA level, and cyclin D1 expression; 2) 59.4% of colorectal adenocarcinomas expressed cyclin D1 and its expression was significantly correlated with favorable clinicopathologic variables such as absence of recurrence and/or metastasis; 3) expression of cyclin D1 was associated with a significantly longer overall survival and relapse-free survival in colorectal adenocarcinoma patients. Though expression of β-catenin was also a favorable prognostic factor of colorectal adenocarcinoma by univariate analysis, it was not an independent prognostic indicator by multivariate analysis. It may be due to high correlation between cyclin D1 expression and β-catenin expression. Taken together, our findings indicate that cyclin D1 and β-catenin may be significant favorable prognostic indicators for colorectal adenocarcinoma patients.

β-Catenin is present in different intracellular localizations that are associated with its distinct functions. β-Catenin is part of adherence junctions when bound to the cell membrane [5], whereas it interacts with DNA-binding proteins of the T-cell factor/lymphoid enhancer factor family and acts as a transcriptional activator when located in the nucleus [6,7]. It is well known that the nuclear localization of β-catenin is partially regulated by the WNT signaling pathway. In the presence of WNT signaling, multiprotein complexes such as APC,

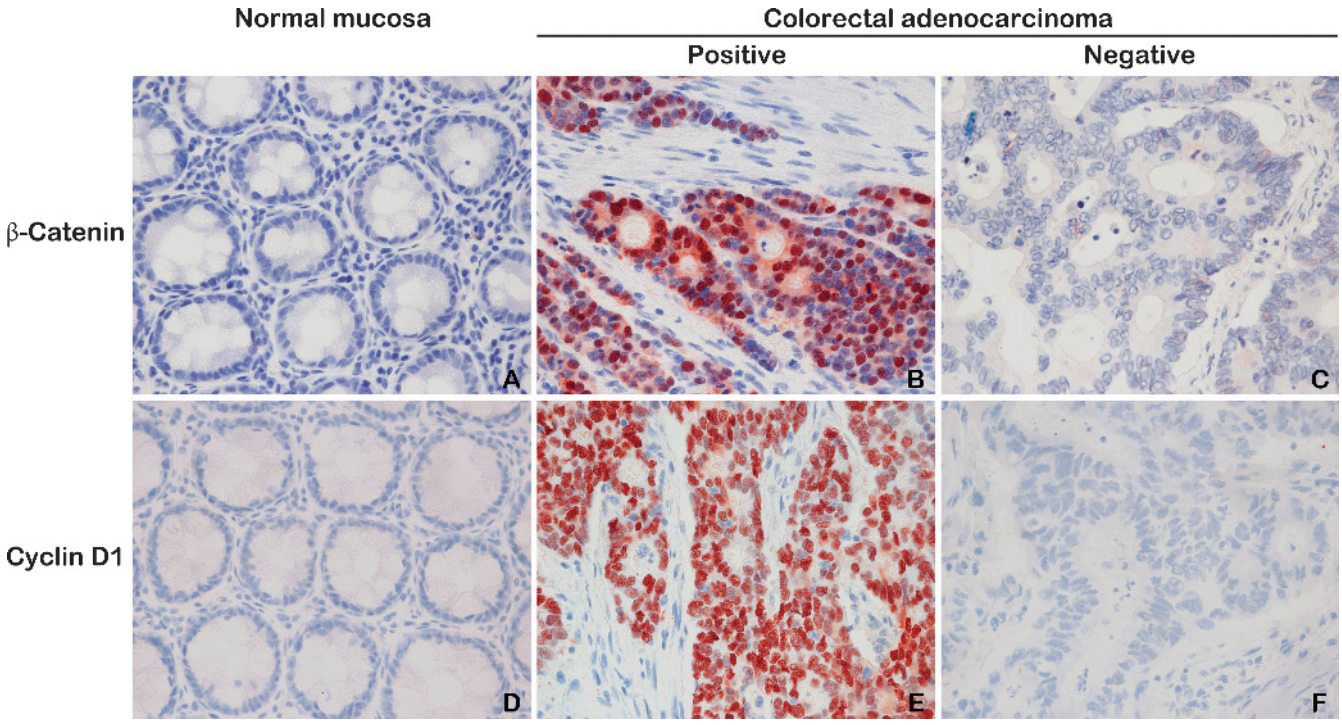

Figure 1. Immunohistochemical staining for β-catenin (A–C) and cyclin D1 (D–F) in normal colorectal mucosa and colorectal adenocarcinoma. Normal mucosa is negative for β-catenin (A) and cyclin D1 (D). In colorectal adenocarcinoma specimens, β-catenin is positive in the nuclei and cytoplasm (B), whereas cyclin D1 show nuclear positivity (E). Some colorectal adenocarcinomas are negative for β-catenin (C) and cyclin D1 (F). Original magnification, ×400.

Table 2. Clinicopathologic Factors and Their Effects on Overall Survival and Relapse-free Survival by Univariate Cox Proportional Hazard Regression Analysis.

	No. of Cases	Overall Survival		Relapse-free Survival	
		HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Overall colorectal adenocarcinoma (*n* = 220)					
Age					
≤65 years	118	1		1	
>65 years	102	1.895 (1.175–3.057)	0.009	1.194 (0.717–1.990)	0.496
Gender					
Male	136	1		1	
Female	84	0.861 (0.527–1.407)	0.550	0.935 (0.561–1.558)	0.797
Location					
Rt. colon	52	1			
Lt. colon	64	0.445 (0.212–0.936)	0.033	0.669 (0.318–1.406)	0.228
Rectum	104	1.003 (0.580–1.737)	0.990	1.073 (0.582–1.975)	0.822
Tumor size					
≤4.0 cm	83	1		1	
>4.0 cm	137	2.006 (1.172–3.434)	0.011	2.419 (1.331–4.398)	0.004
Differentiation					
Well/Moderate	201	1		1	
Poor	19	3.324 (1.741–6.347)	<0.001	2.695 (1.321–5.497)	0.006
Lymphovascular invasion					
Absent	172	1		1	
Present	48	1.624 (0.965–2.732)	0.068	2.053 (1.210–3.482)	0.008
Lymph node metastasis					
Absent	125	1		1	
Present	91	2.410 (1.485–3.909)	<0.001	2.242 (1.337–3.758)	0.002
Recurrence/Metastasis					
No	159	1			
Yes	61	6.735 (4.089–11.093)	<0.001		
Stage					
I and II	122	1		1	
III and IV	98	2.836 (1.719–4.680)	<0.001	3.485 (2.009–6.047)	<0.001
Adjuvant chemotherapy					
No	111	1		1	
Yes	109	0.889 (0.554–1.426)	0.625	1.199 (0.723–1.988)	0.482
Preoperative CEA					
≤5.0 ng/ml	143	1		1	
>5.0 ng/ml	68	2.175 (1.331–3.553)	0.002	2.524 (1.507–4.227)	<0.001
Preoperative CA19-9					
≤37.0 U/ml	138	1		1	
>37.0 U/ml	21	3.778 (2.088–6.833)	<0.001	4.070 (2.174–7.620)	<0.001
β-Catenin					
Negative	60	1		1	
Positive	158	0.558 (0.339–0.918)	0.022	0.660 (0.387–1.128)	0.129
Cyclin D1					
Negative	88	1		1	
Positive	129	0.494 (0.306–0.796)	0.004	0.491 (0.296–0.813)	0.006
Patients who received adjuvant chemotherapy (*n* = 109)					
β-Catenin					
Negative	30	1		1	
Positive	79	0.440 (0.224–0.866)	0.018	0.597 (0.295–1.208)	0.151
Cyclin D1					
Negative	42	1		1	
Positive	66	0.261 (0.131–0.521)	<0.001	0.474 (0.241–0.932)	0.030

axin, and glycogen synthase kinase 3β that are usually responsible for the cytoplasmic degradation of β-catenin become inactive [4,23,24]. As a result, β-catenin is accumulated in the cytoplasm and can be translocated into the nucleus where it leads to subsequent activation of cyclin D1. The localization of β-catenin by immunohistochemistry in CRC has shown wide variability in terms of proportion with nuclear, cytoplasmic, or membranous staining [25,26]. In this study, we considered nuclear expression of β-catenin as positive staining and this is reasonable because β-catenin plays the transcriptional role in the nucleus. In several previous studies, the results of correlation of β-catenin expression and colon cancer prognosis or progression were inconsistent. Hugh et al. [11] reported that nuclear expression of β-catenin was independently predictive of a shorter survival. Chung et al. [27] reported that nuclear expression of β-catenin did not have a significant correlation with survival of CRC, whereas nuclear expression of phosphor–β-catenin was associated with a better prognosis. Horst et al. [28] reported that colon cancers with no nuclear β-catenin expression had the worst prognosis and this is concordant with our result. Moreover, nuclear expression of β-catenin has been reported to be correlated with good prognosis in hepatocellular carcinoma and ovarian carcinoma [29,30]. These findings also support that nuclear expression of β-catenin is associated with good prognosis as in our result.

Cyclin D1 overexpression in tumors can be acquired by several mechanisms such as *CCLND1* gene amplification, increased transcription by constitutive activation of transcriptional regulators, or increased mRNA or protein stability [31]. Though there were several previous studies that have examined the relationship between cyclin D1 expression and clinical outcome in colon cancer [16–22], they have yielded

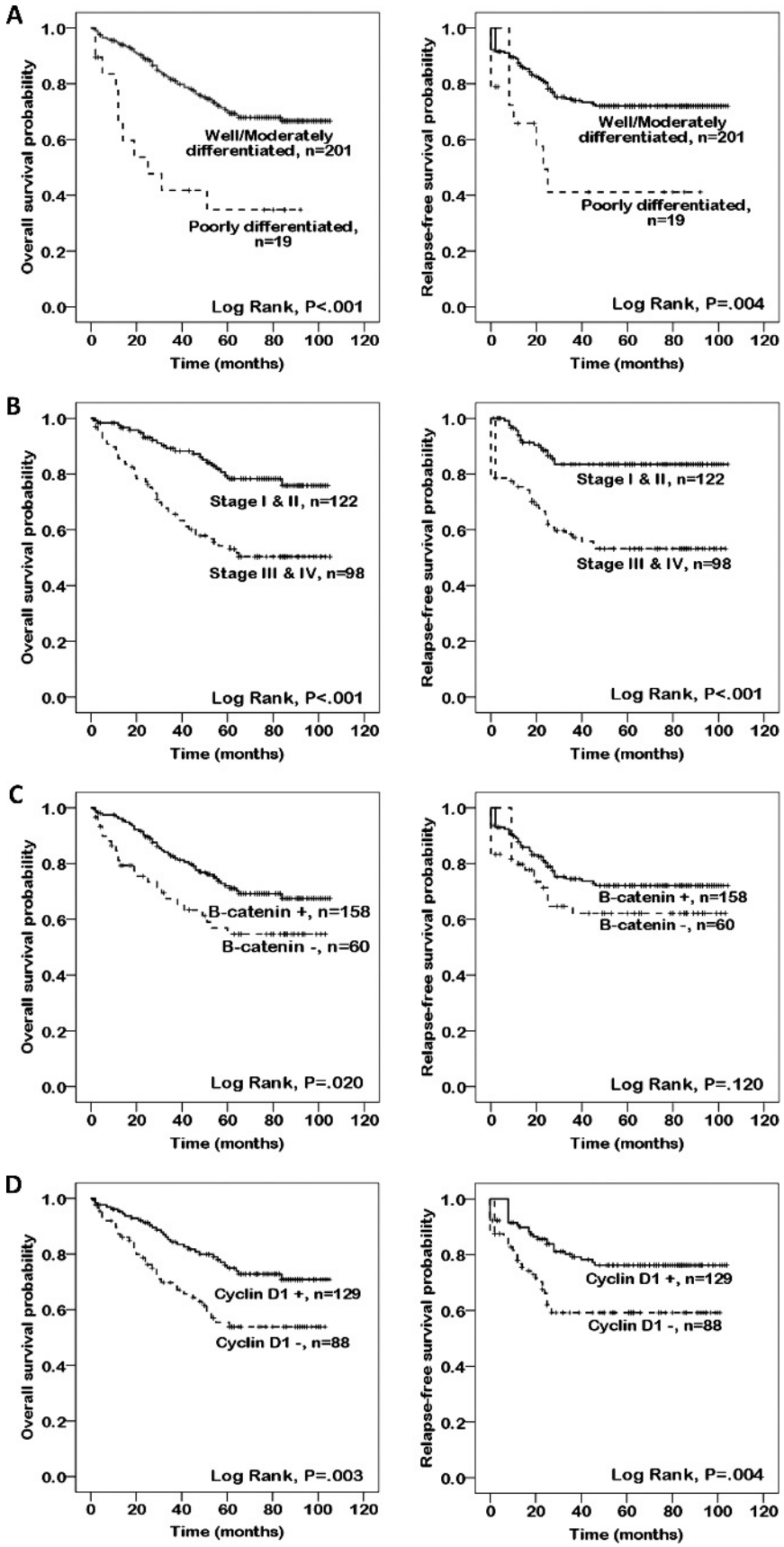

Figure 2. Survival analysis in colorectal adenocarcinoma. (A) Overall survival and relapse-free survival in well/moderately differentiated and poorly differentiated groups. (B) Overall survival and relapse-free survival in low clinical stage (I and II) and high clinical stage (III and IV) groups. (C) Relationship of nuclear β-catenin expression to overall survival and relapse-free survival. (D) Relationship of nuclear cyclin D1 expression to overall survival and relapse-free survival. *P* values were determined by comparing survival distributions using the log-rank test.

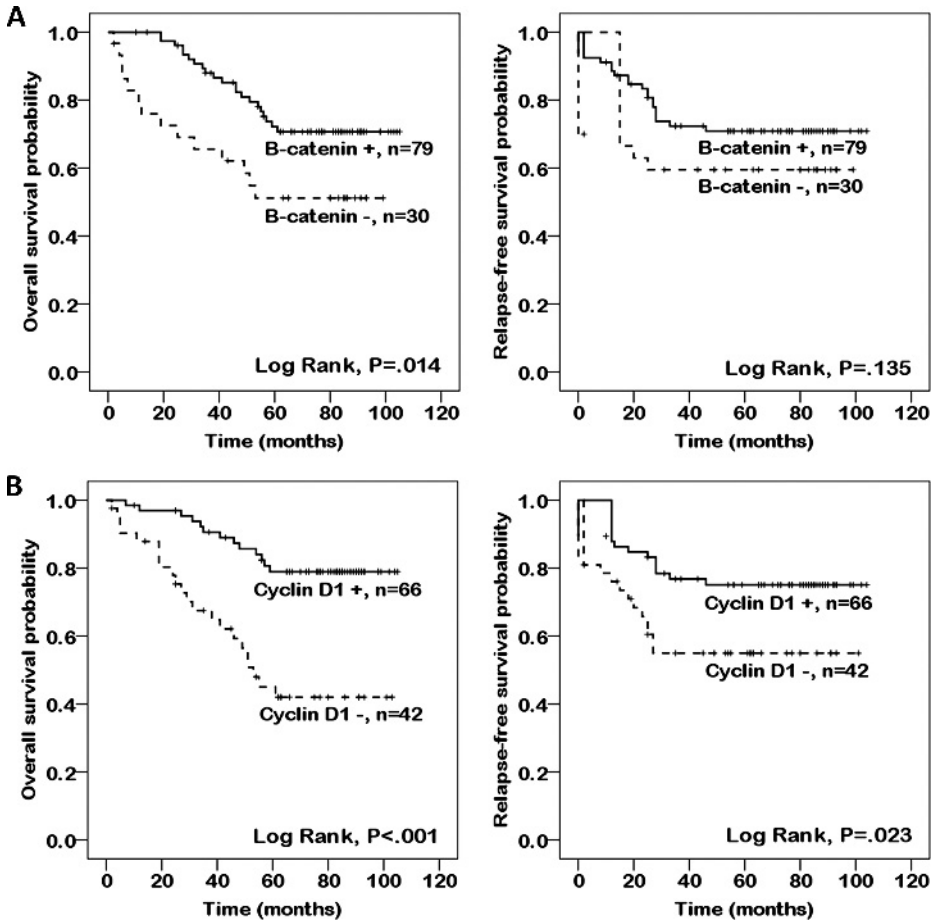

Figure 3. Survival analysis in colorectal adenocarcinoma patients who had received adjuvant chemotherapy. (A) Relationship of nuclear β-catenin expression to overall survival and relapse-free survival. (B) Relationship of nuclear cyclin D1 expression to overall survival and relapse-free survival. *P* values were determined by comparing survival distributions using the log-rank test.

Table 3. Clinicopathologic Factors and Their Effects on Overall Survival and Relapse-free Survival by Multivariate Cox Proportional Hazard Regression Analysis.

	Overall Survival		Relapse-free Survival	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Overall colorectal adenocarcinoma (*n* = 156)				
Age				
≤65 years	1		1	
>65 years	2.185 (1.264–3.777)	0.005	1.012 (0.561–1.825)	0.967
Differentiation				
Well/Moderate	1		1	
Poor	2.690 (1.233–5.869)	0.013	1.753 (0.720–4.270)	0.216
Stage				
I and II	1		1	
III and IV	2.540 (1.388–4.649)	0.003	2.857 (1.421–5.745)	0.003
Preoperative CA19-9				
≤37.0 U/ml	1		1	
>37.0 U/ml	3.182 (1.661–6.096)	<0.001	1.764 (0.786–3.959)	0.169
Cyclin D1				
Negative	1		1	
Positive	0.477 (0.279–0.817)	0.007	0.508 (0.282–0.916)	0.024
Patients who received adjuvant chemotherapy (*n* = 82)				
β-Catenin				
Negative	1		1	
Positive	0.406 (0.194–0.850)	0.017	1.167 (0.478–2.847)	0.734
Cyclin D1				
Negative	1		1	
Positive	0.205 (0.092–0.457)	<0.001	0.452 (0.212–0.963)	0.040

Variables include age, sex, location, tumor size, differentiation, lymphovascular invasion, stage, preoperative CEA level, preoperative CA19-9 level, β-catenin expression, and cyclin D1 expression.

inconsistent results. Some studies have demonstrated that cyclin D1 expression was associated with poor prognosis [16,17]; however, other studies have shown that cyclin D1 expression was associated with good prognosis as in the present study [18,19]. The other studies demonstrated no prognostic significance of cyclin D1 expression [20–22]. The controversial results in the expression of β-catenin and cyclin D1 may be due to differences in sample sizes, intrinsic tumor heterogeneity, methods of scoring criteria, and antibodies used to access their expression in CRCs.

In general, oncogene activation or tumor suppressor gene inactivation is associated with aggressive tumor behavior. However, this hypothesis is not always true as shown in an example that microsatellite instability, which is known to cause inactivation of tumor suppressor genes, is associated with better patient outcome [32]. Though the reason why cyclin D1 expression is associated with good prognosis is unclear, Ogino et al. [19] have proposed the possibility that cyclin D1–negative cancers might have bypassed the cyclin D1 activation, which might cause more aggressive behavior than cyclin D1–activated cancers through accumulation of multiple genetic and epigenetic events during colorectal carcinogenesis. Moreover, association between cyclin D1 expression and good prognosis have been described in non–small cell lung cancer, breast cancer, and bladder cancer [33–35]. Nosho et al. [36] have reported that cyclin D1 was frequently overexpressed in microsatellite unstable CRCs that is known to have a better prognosis than microsatellite stable CRCs. This finding also supports our result that cyclin D1 overexpression is associated with good prognosis. In addition, when we performed survival analysis in the subgroup of patients according to the treatment modality, the expression of cyclin D1 predicted better overall survival and relapse-free survival in the patients who received adjuvant chemotherapy but not in the patients treated with surgery only. Thus, we can infer that colorectal adenocarcinomas expressing cyclin D1 may predict better response to adjuvant chemotherapy than those that did not. This assumption is compatible with the result of recent study that reported increased chemosensitivity in cyclin D1 overexpressing myeloma cell line [37]. However, more studies are needed to determine the effect of cyclin D1 expression on the chemotherapeutic response in colorectal adenocarcinoma.

In conclusion, expression of β-catenin and cyclin D1 was associated with favorable clinicopathologic variables of colorectal adenocarcinoma and with good prognosis of colorectal adenocarcinoma patients. Although the role of β-catenin and cyclin D1 in tumor progression is not clear, our data suggest that their expression is a clinically significant prognostic indicator for colorectal adenocarcinoma patients.

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