Int J Clin Exp Pathol 2018;11(12):6032-6038
www.ijcep.com /ISSN:1936-2625/IJCEP0086817

Original Article

The expressions of YAP1, β-catenin and survivin in colon cancer tissues and their clinical significance

Qianwen Shao[1], Jing Xu[2], Rong Deng[3], Wei Wei[3], Bing Zhou[3], Chao Yue[3], Miaoling Zhu[4], Haitao Zhu[5]

[1]Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Guangzhou Road 300, Nanjing 210029, Jiangsu Province, China; [2]Genetic Testing Center, Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, Guangzhou Road 300, Nanjing 210029, Jiangsu Province, China; Departments of [3]General Surgery, [4]Pathology, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu Province, China; [5]Colorectal Cancer Center, Department of General Surgery, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Nanjing, Jiangsu Province, China

Received October 16, 2018; Accepted October 26, 2018; Epub December 1, 2018; Published December 15, 2018

Abstract: We aimed to evaluate the effects of YAP1, β-catenin, and survivin on the onset and progression of colon cancer, and to explore their correlations. The expressions of YAP1, β-catenin, and survivin in 106 colon cancer tissues and 55 normal colon mucosa tissues were measured by immunohistochemical assay. The correlations between their expressions and clinical and pathological characteristics were analyzed. The expression rates of YAP1, β-catenin, and survivin in colon cancer tissues were significantly higher than those in normal colon mucosa tissues ($P<0.001$). The expressions of YAP1, β-catenin, and survivin in colon cancer were correlated with neither gender nor age ($P>0.05$), but with the degree of differentiation, depth of invasion, lymph node metastasis and Duke's stage ($P<0.05$). Pearson's correlation analysis showed that the expressions of YAP1, β-catenin, and survivin in colon cancer tissues were all positively correlated ($P<0.05$). The overexpression of YAP1, β-catenin, and survivin played an important role in the onset and progression of colon cancer, providing reference value for prognosis prediction.

Keywords: YAP1, β-catenin, survivin, colon cancer

Introduction

Colon cancer is a common malignancy in the digestive system, the onset and progression of which involve multiple genes, pathways and stages. Although early screening strategies such as colonoscopy, improved surgical methods and postoperative chemotherapy have been widely used in clinical practice, the mortality rate of colon cancer is still high. Research on colon cancer-related genes is expected to reduce the mortality rate. The Wnt signaling pathway plays a vital role in maintaining the characteristics of cancer stem cells. The abnormalities of its oncogenes, anti-oncogenes, and cell adhesion molecules are closely related to the onset and progression of tumors. β-Catenin is a crucial component of the Wnt signaling pathway. After being activated abnormally, it can promote the self-renewal of cancer stem cells and regulate the dysfunction of differentiation. Moreover, it predominantly participates in tumor invasion and metastasis [1]. The Hippo signaling pathway consists of different proteins that control the growth, differentiation and regeneration of various tissues. This is a tumor suppressor pathway, with YAP1 as the core. YAP1 is the downstream signaling molecule of the Hippo pathway. Under normal conditions, YAP1 is not or lowly expressed. Some molecules in this pathway inhibit YAP1 expression through a series of phosphorylations. Once the upstream or downstream molecules of the pathway are mutated, molecules that are not or lowly expressed are hyperactivated, thereby accelerating the proliferation of cells and forming tumors. YAP1 plays key roles in human development, growth, DNA repair and endogenous homeostasis. After being normally activated, YAP1 promotes tissue proliferation, differentiation and regeneration, exerting positive effects on wound repair in organisms. Upon hyperactivation, however, it evidently facilitates the proliferation of cells and the formation of

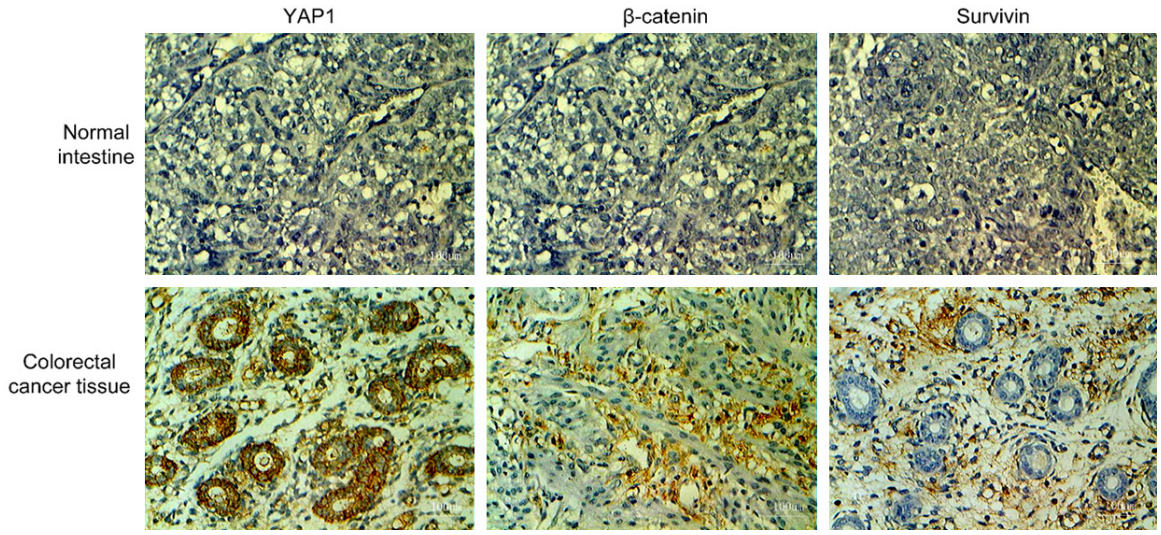

Figure 1. Expressions of YAP1, β-catenin and survivin in colon cancer tissues and normal colon mucosa tissues.

tumors [2]. Survivin, as a bifunctional protein, can inhibit cell apoptosis, promote proliferation and metastasis, and regulate mitosis. The survivin gene is not expressed in normal tissues, but it is positively expressed in almost all tumor tissues that have been studied. Therefore, high expression of survivin is a marker for most tumors. β-Catenin accumulates in the nucleus after abnormal activation of the Wnt signaling pathway, binds YAP1, and activates the expression of the downstream target gene survivin, leading to malignant transformation [3]. In this study, we detected the expressions of YAP1, β-catenin and survivin in colon cancer and normal colon mucosa tissues by immunohistochemical assay. The clinical and pathological characteristics were analyzed to explore the correlations with these expressions, aiming to provide a theoretical basis for the innovative diagnosis and treatment of colon cancer.

Materials and methods

Baseline clinical data

A total of 106 primary colon cancer tissues were collected from patients who received surgical resections from June 2016 to January 2018 in our hospital. There were 64 males and 42 females, with similar ages (P>0.05). None of the patients had received radiotherapy, chemotherapy, or other special treatment for their tumors before the surgery, and the cancer was pathologically confirmed after the surgery. Patients with a family history of colon cancer were excluded. Meanwhile, 55 normal colon mucosa tissues located more than 5 cm away from the edge of colon cancer were used as controls.

Main reagents

Mouse anti-human β-catenin monoclonal antibody SC-7963 was purchased from Santa Cruz (USA). Rabbit anti-human YAP1 and β-catenin polyclonal antibodies were bought from Abcam (UK). An immunohistochemical Envision two-step staining kit and citrate antigen retrieval solution (pH 6.0) were obtained from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (China). A DAB color development kit was provided by Fuzhou Maixin Biotechnology Development Co., Ltd. (China).

Immunohistochemical staining

All tissue samples were fixed in 10% neutral formalin, embedded in paraffin, and routinely sectioned into 4 μm-thick slices for HE and immunohistochemical staining, respectively. Immunohistochemical staining (using the EnVision two-step method) was performed according to the kit's instructions, using known positive sections as a positive control and PBS instead of primary antibody as a negative control.

Determination of results

YAP1 and survivin stained as brownish yellow particles in the cell nucleus were determined to be positive signals. Five representative high-power fields were selected for each sample. Percentage of positive cells: ≤10%, 1 point;

Table 1. Expressions of YAP1, β-catenin and survivin in colon cancer tissues and normal colon mucosa tissues

Group	Case No.	YAP1 expression		χ^2 value	*P* value	β-Catenin expression		χ^2 value	*P* value	Survivin expression		χ^2 value	*P* value
		Positive	Positive rate (%)			Positive	Positive rate (%)			Positive	Positive rate (%)		
Normal colon mucosa tissue	55	9	16.36	23.94	<0.001	0	0.00	Fisher's exact test	<0.001	8	14.55	39.84	<0.001
Colon cancer tissue	106	60	56.60			54	50.94			71	66.98		

Table 2. Correlations between YAP1, β-catenin and survivin expressions in colon cancer tissues and clinical and pathological characteristics

Item	Case No.	YAP1		χ^2 value	*P* value	β-Catenin		χ^2 value	*P* value	Survivin		χ^2 value	*P* value
		Positive	Positive rate (%)			Positive	Positive rate (%)			Positive	Positive rate (%)		
Age (year)													
<60	31	16	51.61	0.44	0.505	13	41.94	1.422	0.233	20	64.52	0.12	0.729
≥60	75	44	58.67			41	54.67			51	68.00		
Gender													
Male	64	36	56.25	0.08	0.928	33	51.56	0.02	0.875	47	73.44	3.04	0.081
Female	42	24	57.14			21	50.00			24	57.14		
Differentiation degree													
High	26	8	30.77	15.32	0.004	5	19.23	36.11	0.000	9	34.62	28.86	0.000
Moderate	68	43	63.24			49	72.06			52	76.47		
Low	12	9	75.00			10	83.33			10	83.33		
Invasion depth													
T1~T2	35	14	40.00	5.86	0.015	5	14.29	28.10	0.000	15	42.86	8.38	0.038
T3~T4	71	46	64.79			49	69.01			51	71.83		
Lymph node metastasis													
No	50	19	38.00	13.33	0.003	8	16.00	46.24	0.000	25	50.00	12.34	0.000
Yes	56	41	73.21			46	82.14			46	82.14		
Duke's stage													
A + B	29	21	72.41	4.06	0.044	10	34.48	4.328	0.038	23	79.31	4.94	0.026
C + D	77	39	50.45			44	57.14			43	55.84		

Table 3. Correlations of YAP1 expression with β-catenin and survivin expressions in colon cancer tissues

Item	YAP1		r value	*P* value
	+	-		
β-Catenin				
+	46	8	0.342	<0.001
-	14	38		
Survivin				
+	54	17	0.485	<0.001
-	6	29		

Table 4. Correlations between β-catenin and survivin expressions

Item	β-Catenin		r value	*P* value
	+	-		
Survivin				
+	49	22	0.541	<0.001
-	5	30		

11%-50%, 2 points; 51%-75%, 3 points; >75%, 4 points. Staining intensity: nonspecific staining, 0 point; light brown, 1 point; brownish yellow, 2 points; brown, 3 points. The percentage of positive cells was multiplied with staining intensity, with the product of ≥2 points as positive. β-Catenin is expressed normally on the cell membrane and in the cytoplasm, but abnormally in the nucleus. The results were evaluated according to a previous study [4], and 500 cells were randomly counted under a high-power microscope. When >5% of the cells showed abnormal nuclear staining, positive expression was determined.

Statistical analysis

All data were analyzed by SPSS16.0. The numerical data were compared with the χ^2 test and Fisher's exact test. The categorical data were expressed as the mean ± standard deviation. Inter-group comparisons were conducted by the t test. Correlations were studied by Pearson's correlation analysis. P<0.05 was considered statistically significant.

Results

Expressions of YAP1, β-catenin and survivin in colon cancer tissues and normal colon mucosa tissues

The expression rates of YAP1, β-catenin and survivin in colon cancer tissues were significantly higher than those in normal colon mucosa tissues (56.60% vs. 16.36%, 50.94% vs. 0.00%, 56.60% vs. 16.36%; P<0.001) (**Figure 1** and **Table 1**).

Correlations between YAP1, β-catenin and survivin expressions in colon cancer tissues and clinical and pathological characteristics

Of the 106 enrolled cases, 31 were younger than 60 years old and 75 were aged ≥60. The positive expression rates of YAP1 were 51.61% and 58.67% respectively, those of β-catenin were 41.94% and 54.67% respectively, and those of survivin were 64.52% and 68.00% respectively, all without significant differences (P>0.05).

The positive expression rates of YAP1, β-catenin and survivin in males were 56.25%, 51.56% and 73.44% respectively, and those of females were 57.14%, 50.00% and 57.14% respectively, also without significant differences (P>0.05).

There were 26, 68, and 12 highly, moderately, and lowly differentiated cases respectively. The positive expression rates of YAP1 were 30.77%, 63.24% and 75.00% respectively, those of β-catenin were 19.23%, 72.06% and 83.33% respectively, and those of survivin were 34.62%, 76.47% and 83.33% respectively, which all significantly decreased with an increasing degree of differentiation (P<0.05).

There were 35 and 71 cases with invasion depths of T1~T2 and T3~T4 respectively. The positive expression rates of YAP1, β-catenin and survivin in the cases with T1~T2 invasion depth were 40.00%, 14.29% and 42.86% respectively, and those in the cases with T3~T4 invasion depth were 64.79%, 69.01% and 71.83% respectively, with significant differences (P<0.05).

The positive expression rates of YAP1, β-catenin and survivin in cases without lymph node metastasis (n=50) were 38.00%, 16.00% and 50.00% respectively, and the rates of the cases with lymph node metastasis were 73.21%, 82.14%, and 82.14% respectively, also with significant differences (P<0.05).

There were 29 cases at Duke's stages A and B, and 77 cases at stages C and D. The positive expression rates of YAP1, β-catenin and survivin in the cases at stages A and B were

72.41%, 34.48% and 79.31% respectively, and those of the cases at stages C and D were 50.45%, 57.14% and 55.84% respectively, which had significant differences (P<0.05) (**Table 2**).

Correlations between YAP1, β-catenin and survivin expressions in colon cancer tissues

Pearson's correlation analysis showed that the expressions of YAP1 and β-catenin in colon cancer tissues were positively correlated (r= 0.342, P<0.001). Also, the expressions of YAP1 and survivin in the colon cancer tissues were also positively correlated (r=0.485, P<0.001) (**Table 3**).

A Pearson's correlation analysis revealed that the β-catenin and survivin expressions in colon cancer tissues were positively correlated (r=0.541, P<0.001) (**Table 4**).

Discussion

Under normal conditions, YAP1 is highly conservative, maintaining a stable internal environment and a dynamic balance between cell growth and apoptosis, thereby inhibiting the excessive growth of tissues and organs and preventing the onset and progression of tumors. When hyperactivated, however, YAP1 excessively promotes cell proliferation and tumor formation. High YAP1 expression may enhance tumor invasion and metastasis and facilitate tumor cells' destruction of the extracellular matrix and basilar membrane. Xiao et al. reported that a high YAP1 expression elevated the invasion and metastasis capacities of tumor cells [6]. In addition, Avruch et al. found that the overgrowth and strong invasion of colon cancer cells may be related to high YAP1 expression [7]. In colon cancer cells, the phosphorylation and degradation of β-catenin are blocked, so they continuously accumulate in the cytoplasm, then they enter the cell nucleus to initiate the expressions of downstream target genes. Therefore, β-catenin is abnormally expressed in the cytoplasm or nucleus of colon cancer tissues to various extents. Moreover, Hao et al. reported that abnormal β-catenin expression was a common event during the onset and progression of colorectal adenoma, also probably as an early event [8]. As a member of the inhibitor of apoptosis protein family, survivin is the strongest anti-apoptotic factor found so far [9]. Survivin is specifically expressed in the G2/M phase of the cell cycle, which participates in gene transcription by binding the microtubules of mitotic spindles, then attenuating abnormal proliferation and differentiation [10]. It has previously been reported that survivin was widely expressed in most cancers such as gastric cancer, breast cancer and lung cancer, but not in normal tissues [11-13]. Similarly, in this study, the expression rates of YAP1, β-catenin and survivin in colon cancer tissues were significantly higher than those in normal colon mucosa tissues (P<0.05), indicating that they were all involved in the onset and progression of colon cancer. Their high expressions induced disorders of apoptosis regulation, thus facilitating abnormal cell proliferation and malignant transformation.

Liu et al. reported that the YAP1 expression in cervical squamous cell carcinoma was significantly higher than it was in normal tissue, being positively correlated with differentiation degree, lymph node metastasis, and early recurrence [14]. Meanwhile, Su et al. found that the YAP1 expression in non-small-cell lung carcinoma tissue was closely correlated with the stage [15]. As to the relationship among β-catenin, invasion, and metastasis, Wang et al. reported that patients with colon cancer hepatic metastasis had a higher abnormal expression rate of β-catenin than that of patients without metastasis, and the abnormal expressions in primary and metastatic foci were positively correlated [16]. Additionally, Ma et al. found that abnormal β-catenin expression was only negatively correlated with the differentiation degree of colon cancer [17]. Gu et al. proved that the ectopic expression of β-catenin was closely correlated with tumor invasion depth and metastasis [18]. The differences between the results were linked to case selection, immunohistochemical method, antibody origin, and determination criteria for immunostaining. Bànkfalvi et al. found that β-catenin had strong positive expressions mainly in normal mammary lumens, basal cells, benign hyperplastic tissues, and early tumor lesions in glandular tubes, accompanied by significantly down-regulated expressions in lymph node metastasis foci [19]. Moreover, Brabletz et al. verified that the nuclear expression of β-catenin was related to the lymph node metastasis and Duke's stage of colon cancer [20].

Collectively, abnormal β-catenin expression is correlated with poor tumor differentiation, high stage, and lymph node and distal metastasis. The tumors are prone to invading surrounding tissues and dissociating primary foci, suggesting that abnormal β-catenin expression is correlated with tumor invasion and metastasis. Possibly, β-catenin is translocated from the cell membrane, which induces the dissociation of β-catenin-E-cadherin complex and the reduction of adhesion ability between the same type of cells, thereby enhancing tumor cell metastasis [21]. Until now, the relationships between positive survivin expression and clinical and pathological factors in different tumors remain controversial [22]. In this study, the expressions of YAP1, β-catenin and survivin in colon cancer were correlated with neither gender nor age ($P>0.05$), but with the degree of differentiation, depth of invasion, lymph node metastasis, and Duke's stage ($P<0.05$). Hence, their expressions increased with the aggravation of colon cancer, also playing crucial regulatory roles in invasion and lymph node metastasis processes.

Pearson's correlation analysis revealed that the expressions of YAP1, β-catenin and survivin in colon cancer tissues were all positively correlated ($P<0.05$). Accordingly, we postulated that β-catenin and YAP1 formed a transcription complex in the nucleus during the onset of colon cancer, then activating the expression of the downstream target gene survivin. In other words, they worked synergistically.

Conclusion

In summary, YAP1, β-catenin, and survivin synergistically participated in the onset, progression and recurrence of colon cancer. Clarifying the relationships between them and the underlying mechanism provides valuable evidence for the clinical treatment and prognosis prediction of this cancer.

Acknowledgements

This study was financially supported by the Key Project of and Technology Department of Jiangsu Province of China (no. BL2014092), the Provincial Natural Science Foundation (no. BK20131438), the Six Talent Peaks Project in Jiangsu Province (no. WSW-038) and the Key Project of Cancer Hospital of Nanjing Medical University.

Disclosure of conflict of interest

None.

Address correspondence to: Haitao Zhu, Colorectal Cancer Center, Department of General Surgery, Jiangsu Cancer Hospital & Jiangsu Institute of Cancer Research & The Affiliated Cancer Hospital of Nanjing Medical University, Baiziting 42, Nanjing 210009, Jiangsu Province, China. Tel: +86-25-86-83284711; E-mail: zhuhtchnmu@yeah.net

References

[1] Zhang Z, Zhou C, Chang Y, Zhang Z, Hu Y, Zhang F, Lu Y, Zheng L, Zhang W, Li X and Li X. Long non-coding RNA CASC11 interacts with hnRNP-K and activates the WNT/β-catenin pathway to promote growth and metastasis in colorectal cancer. Cancer Lett 2016; 376: 62-73.

[2] White BD, Chien AJ and Dawson DW. Dysregulation of Wnt/β-catenin signaling in gastrointestinal cancers. Gastroenterology 2012; 142: 219-232.

[3] Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, Montgomery EA and Anders RA. Expression of yes-associated protein in common solid tumors. Hum Pathol 2008; 39: 1582-1589.

[4] Nguyen HT, Kugler JM, Loya AC and Cohen SM. USP21 regulates Hippo pathway activity by mediating MARK protein turnover. Oncotarget 2017; 8: 64095-64105.

[5] Zhang J, Ji JY, Yu M, Overholtzer M, Smolen GA, Wang R, Brugge JS, Dyson NJ and Haber DA. YAP-dependent induction of amphiregulin identifies a non-cell-autonomous component of the Hippo pathway. Nat Cell Biol 2009; 11: 1444-1450.

[6] Xiao L, Zhou H, Li XP, Chen J, Fang C, Mao CX, Cui JJ, Zhang W, Zhou HH, Yin JY and Liu ZQ. MicroRNA-138 acts as a tumor suppressor in non small cell lung cancer via targeting YAP1. Oncotarget 2016; 7: 40038-40046.

[7] Avruch J, Zhou D and Bardeesy N. YAP oncogene overexpression supercharges colon cancer proliferation. Cell Cycle 2012; 11: 1090-1096.

[8] Hao X, Tomlinson I, Ilyas M, Palazzo JP and Talbot IC. Reciprocity between membranous and nuclear expression of β-catenin in colorectal tumours. Virchows Arch 1997; 431: 167-172.

[9] Toi M, Hoshina S, Takayanagi T and Tominaga T. Association of vascular endothelial growth factor expression with tumor angiogenesis and with early relapse in primary breast cancer. Cancer Sci 1994; 85: 1045-1049.

[10] Giodini A, Kallio MJ, Wall NR, Gorbsky GJ, Tognin S, Marchisio PC, Symons M and Altieri

DC. Regulation of microtubule stability and mitotic progression by survivin. Cancer Res 2002; 62: 2462-2467.
[11] Wakana Y, Kasuya K, Katayanagi S, Tsuchida A, Aoki T, Koyanagi Y, Ishii H and Ebihara Y. Effect of survivin on cell proliferation and apoptosis in gastric cancer. Oncol Rep 2002; 9: 1213-1218.
[12] Tanaka K, Iwamoto S, Gon G, Nohara T, Iwamoto M and Tanigawa N. Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. Clin Cancer Res 2000; 6: 127-134.
[13] Kren L, Brazdil J, Hermanova M, Goncharuk VN, Kallakury BV, Kaur P and Ross JS. Prognostic significance of anti-apoptosis proteins survivin and bcl-2 in non-small cell lung carcinomas: a clinicopathologic study of 102 cases. Appl Immunohistochem Mol Morphol 2004; 12: 44-49.
[14] Liu T, Liu Y, Gao H, Meng F, Yang S and Lou G. Clinical significance of yes-associated protein overexpression in cervical carcinoma: the differential effects based on histotypes. Int J Gynecol Cancer 2013; 23: 735-742.
[15] Su LL, Ma WX, Yuan JF, Shao Y, Xiao W and Jiang SJ. Expression of Yes-associated protein in non-small cell lung cancer and its relationship with clinical pathological factors. Chi Med J 2012; 125: 4003-4008.
[16] Wang L, Cheng H, Liu Y, Wang L, Yu W, Zhang G, Chen B, Yu Z and Hu S. Prognostic value of nuclear β-catenin overexpression at invasive front in colorectal cancer for synchronous liver metastasis. Ann Surg Oncol 2011; 18: 1553-1559.
[17] Ma SP, Shan JX and Wang H. Expression of E-cadherin and β-catenin in colorectal carcinoma and relationship with prognosis. J Pract Oncol 2003; 17: 268-270.
[18] Gu GL, Wang SL, Wei XM, Ren L, Xiong M, Hu YY, Li DC, Zou FX and Cheng J. Cyclooxgenase-2, β-catenin and matrix metalloproteinase-7 expression and their correlation with invasion/metastasis behaviours of hereditary nonpolyposis colorectal cancer. World Chin J Digestol 2009; 17: 151-157.
[19] Bànkfalvi A, Terpe HJ, Breukelmann D, Bier B, Rempe D, Pschadka G, Krech R, Lellè RJ and Boecker W. Immunophenotypic and prognostic analysis of E-cadherin and β-catenin expression during breast carcinogenesis and tumour progression: a comparative study with CD44. Histopathology 1999; 34: 25-34.
[20] Brabletz T, Jung A and Kirchner T. β-Catenin and the morphogenesis of colorectal cancer. Virchows Arch 2002; 441: 1-11.
[21] Bonitsis N, Batistatou A, Karantima S and Charalabopoulos K. The role of cadherin/catenin complex in malignant melanoma. Exp Oncol 2006; 28: 187-193.
[22] Nagaraj AB, Joseph P, Kovalenko O, Singh S, Armstrong A, Redline R, Resnick K, Zanotti K, Waggoner S and DiFeo A. Critical role of Wnt/β-catenin signaling in driving epithelial ovarian cancer platinum resistance. Oncotarget 2015; 6: 23720-23734.