Int J Clin Exp Pathol 2019;12(10):3928-3933
www.ijcep.com /ISSN:1936-2625/IJCEP0100707

*Original Article*

# Correlation between CK1α and β-catenin Ser45-phosphorylation in patients with esophageal squamous cell carcinoma

Xinning Pan[1], Lili Ma[2], Jinsheng Wang[1]

*[1]Department of Pathology, Changzhi Medical College, Changzhi, Shanxi, China; [2]Department of Pathology, Heping Hospital Affiliated to Changzhi Medical College, Changzhi, Shanxi, China*

Received August 7, 2019; Accepted August 28, 2019; Epub October 1, 2019; Published October 15, 2019

**Abstract:** Wnt/β-catenin signaling plays important role in development and tumorigenesis. The accumulation of β-catenin in cytoplasm/nucleus leads to many diseases including cancer. In the normal cells, β-catenin is phosphorylated by CKIα and GSK-3, then phosphorylated β-catenin is sequentially degraded. However, the exact role and correlation of CK1α and β-catenin Ser45-phosphorylation in esophageal squamous cell carcinoma (ESCC) remains little known. Purpose: The present study was aimed at exploring the role and the correlation between the expression of CK1α and β-catenin Ser45-phosphorylation in ESCC. Methods: The expression of CK1α and β-catenin Ser45-phosphorylation were detected by immunohistochemical technique in 90 cases of ESCC and their corresponding adjacent nonneoplastic esophageal tissues (n=90), and evaluated the correlation between the expression of CK1α, phosphorylation at Ser45 of β-catenin and clinicopathological parameters of ESCC patients. Results: The lever of the expression of CK1α in ESCC was 63.3% (57/90), significantly lower than that in nonneoplastic esophageal tissues was 84.4% (76/90), ($X^2$=16.567, P=0.000). The lever of the expression of phosphorylation at Ser45 of β-catenin in ESCC was 65.6% (59/90), significantly lower than that in nonneoplastic esophageal tissues was 88.9% (80/90), ($X^2$=10.340, P=0.003). The expression of CK1α and β-catenin Ser45-phosphorylation was significantly related to the degree of tumor cell differentiation, but not with age, gender, tumor size, AJCC clinical stage and lymphatic metastasis. And CK1α and β-catenin Ser45-phosphorylation expression were also positively correlated (0.356, P=0.001). Conclusion: CK1α and β-catenin Ser45-phosphorylation may play an important role in the pathogenesis and development of ESCC, and provide clinically useful information.

**Keywords:** CK1α, β-catenin, β-catenin Ser45-phosphorylation, esophageal squamous cell carcinoma (ESCC), immunohistochemistry (IHC)

## Introduction

Esophageal cancer (EC) is the eighth most common cancer worldwide, with an estimated 456,000 new cases diagnosed per year, and it is the malignancy with the sixth highest mortality rate [1, 2]. Esophageal squamous cell carcinoma (ESCC) is the major histological subtypes of EC, which is recognized as a prevalent cancer with a high morbidity rate in China, especially in Taihang Mountain [3]. The current treatment of ESCC still is not effective because of invasion and migration. Therefore, deepening the understanding of the pathogenesis of EC and Identifying novel targeted therapeutic strategies in ESCC are urgently needed.

CK1 (casein kinase 1) family is a serine/threonine protein kinase, ubiquitously expressed in eukaryotic organism [4]. Seven family members were identified, including CK1α, CK1β, CK1γ1, CK1γ2, CK1γ3, CK1δ, and CK1ε [5]. CK1 members are involved in many cellular processes. And CK1 isoforms are the key regulators of several cellular growth and processes, including cell cycle control, DNA repair, Wnt, p53 and Hedgehog signaling [6, 7]. Particularly, CK1α was reported to phosphorylates a large number of cellular protein. In the Wnt/β-Catenin signaling pathway, CK1α is a negative regulator by acting as a priming kinase for β-catenin phosphorylation on Ser45, which is essential for further phosphorylations by GSK3β at the Ser/Thr

residues 33, 37 and 41 [8, 9]. Without phosphorylation at Ser45 of β-catenin, β-catenin is not degraded and gets accumulated, which may leads to some cancers. Although CK1α and β-catenin Ser45-phosphorylation play important roles in many tumors pathogenesis, the role and the correlation between the expression of CK1α and β-catenin Ser45-phosphorylation in tumor remain unclear. In the present study, we explored the expression and correlation of CK1α and phosphorylation at Ser45 of β-catenin in ESCC and analyzed the relationship between CK1α, β-catenin Ser45-phosphorylation expression and clinicopathological parameters.

## Material and methods

### *Patients and tissue history*

Tumor tissue samples evaluated in our experiment were obtained from 90 patients (41 female, 49 male; median age 60 years; range 36-76 years) who had undergone radical esophagectomy in the First Affiliated Hospital of Changzhi Medical College (Changzhi, Shanxi, China) from January 2012 to October 2013. The patients were selected at their first diagnosis and none of them received radiotherapy, chemotherapy and/or immunotherapy before the radical surgical treatment. All samples were matched with the corresponding adjacent normal mucosa (>3-10 cm). The sample tissues collected immediately were made into liquid nitrogen snap-frozen specimens and paraffin blocks until use. All samples, with a histopathologic diagnosis of ESCC and the corresponding adjacent normal tissues, were confirmed by two independent pathologists who were blinded to the original diagnosis. No metaplasia, dysplasia, and atypical hyperplasia in the non-neoplastic esophageal tissues, are the strict evaluation criteria. Clinicopathologic data were collected, including age, gender, degree of differentiation, AJCC clinical stage and lymphatic metastasis. Tumors were staged by the American Joint Committee on Cancer (AJCC) staging system [10].

### *Tissue microarray (TMA)*

The tissue microarray was made in collaboration with Shanghai Biochip Company, (Shanghai, China). Mark all cases HE sliced lesions scope and match to the corresponding paraffin wax block. A small core of tissue (2 mm) was drilled out of the original block. This was then transferred to the recipient block. The block was gently warmed to anneal and cooled before the sections are cut. Then, 180 TMA blocks tissues with formalin-fixed, paraffin-embedded (90 case of ESCC and 90 case of corresponding adjacent the corresponding adjacent normal mucosa tissues) were prepared.

### *Immunohistochemistry*

Paraffin sections (4 um thick) obtained from the TMA blocks were cut for the immunohistochemical reactions. The slides were dewaxed, hydrated, then immersed in 3% hydrogen peroxide solution for 10 min. In order to retrieve the antigens, the sections were hyperbaric heated in Ethylene diaminetetra acetic acid (EDTA) buffer (pH 9.0) at 100°C for 8 min. After incubated with 10% normal goat serum at room temperature for 30 min, the slides were incubated with CK1α and β-catenin Ser45-phosphorylation rabbit polyclonal antibody (1:300 dilution, Abcam, Cambridge, UK) overnight at 4°C. After we washed three times with phosphate buffer solution (PBS), the sections were treated with corresponding second antibody (Zhongshan Golden Bridge Corporation, Beijing, China), then treated with peroxidase-conjugated streptavidin. Diaminobenzidine (DAB) were dropped on the sections for color reaction. The gastric carcinoma slides were utlized as positive controls. PBS, as the substitute for primary antibody, was negative controls.

### *Section evaluation of immunohistochemical staining*

A semiquantitative method based on the distribution and intensity of the staining was used to grade CK1α and β-catenin Ser45-phosphorylation immunostaining. Two pathologists without knowledge of the clinical outcomes assessed the degree of immunostaining. When yellow orbrown particles were located in the cell nuclei and (or) cytoplasm, we considered as CK1α and β-catenin Ser45-phosphorylation positive specimen. The intensity of staining was graded as follows: 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). The percentage of positive cells was graded as follows: 1 (0-25%), 2 (26-50%), 3 (51-75%), or 4 (76-100%). The expression of CK1α and phosphorylation at Ser45 of β-catenin were defined

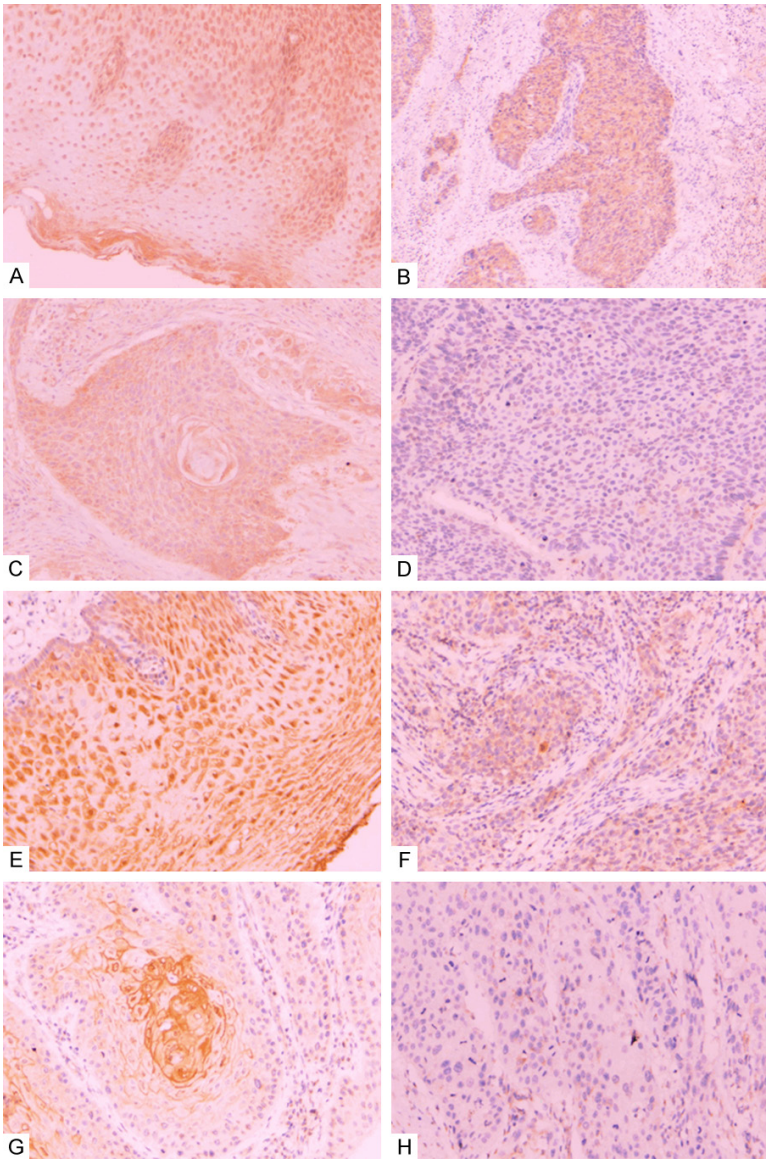


**Figure 1.** A. Expression of CK1α in nonneoplastic esophageal tissue (SP×200); B. Expression of CK1α in ESCC (SP×200); C. Expression of CK1α in high grade ESCC (SP×200); D. Expression of CK1α in low grade ESCC (SP×200). E. Expression of β-catenin Ser45-phosphorylation in nonneoplastic esophageal tissue (SP×200); F. Expression of β-catenin Ser45-phosphorylation in ESCC (SP×200); G. Expression of β-catenin Ser45-phosphorylation in high grade ESCC (SP×200); H. Expression of β-catenin Ser45-phosphorylation in low grade ESCC (SP×200).

according to the final one obtained from the grade of intensity multiplied by the score of percentage of positive cells: negative (0-3), weakly positive (4-5), or strongly positive (6-7) [11].

All analysis was performed with SPSS 18.0 for Windows (SPSS Inc., Chicago, USA). The Chi-square test was used to analyze the correlation between CK1α, β-catenin Ser45-phosphorylation expression and clinicopathological parameters. And then Pearson correlation analysis were performed. A value of $P<0.05$ was considered statistically significant.

## Results

### *Relationship between CK1α, β-catenin Ser45-phosphorylation expression and the clinicopathological parameters of ESCC*

It was found that the CK1α and β-catenin Ser45-phosphorylation expression were in cell cytoplasm, as well as few in nuclei (**Figure 1A**, **1B**, **1E**, **1F**). The CK1α and β-catenin Ser45-phosphorylation expression in ESCC were significantly lower than in the nonneoplastic esophageal tissues. Among 90 primary ESCC, the lever of the expression of CK1α in ESCC was 63.3% (57/90) ($X^2$=16.567, P=0.000), significantly lower than that in nonneoplastic esophageal tissues was 84.4% (76/90). The incidence of a positive expression of Ser45-phosphorylation was 65.6% (59/90), whereas the positive rate of nonneoplastic esophageal tissues was 88.9% (80/90). A significant down-regulation of β-catenin Ser45-phosphorylation immunoreactivity was found between ESCC and the nonneoplastic esophageal tissue ($X^2$=10.340, P=0.003). The statistical analyses also indicated the relationship between CK1α, β-catenin Ser45-phosphorylation expression and clinicopathological parameters. An decreasing trend of CK1α and β-catenin Ser45-phosphorylation expression from high grade to low grade was found, we also demonstrated the significant difference among them (P=0.023) and (P=0.021) (**Figure 1C**, **1D**, **1G**, **1H**). However, no statistically significant correlations be-

**Table 1.** The expression of CK1α and β-catenin Ser45-phosphorylation in ESCC patients with clinicopathological parameters

| Variable | | n | CK1α | | $X^2$ | P | n | P-Ser45-β-catenin | | $X^2$ | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | + | - | | | | + | - | | |
| Overall frequency | Nonneoplastic | 90 | 76 | 14 | 16.567 | 0.000 | 90 | 80 | 10 | 10.340 | 0.003 |
| | ESCC | 90 | 57 | 33 | | | 90 | 59 | 31 | | |
| Gender | Male | 49 | 21 | 28 | 1.775 | 0.183 | 49 | 16 | 33 | 0.361 | 0.548 |
| | Female | 41 | 12 | 29 | | | 41 | 11 | 30 | | |
| Age (yr) at surgery | ≥60 | 58 | 21 | 37 | 0.015 | 0.903 | 58 | 20 | 38 | 1.561 | 0.212 |
| | <60 | 32 | 12 | 20 | | | 32 | 7 | 25 | | |
| Tumor size (cm) | <4 | 54 | 19 | 35 | 0.501 | 0.778 | 54 | 18 | 36 | 2.224 | 0.338 |
| | 4-7 | 32 | 13 | 19 | | | 32 | 7 | 25 | | |
| | ≥8 | 4 | 1 | 3 | | | 4 | 2 | 2 | | |
| Cell differentiation | High-grade | 15 | 11 | 4 | 7.142 | 0.023 | 15 | 10 | 5 | 7.811 | 0.021 |
| | Middle-grade | 64 | 23 | 41 | | | 64 | 16 | 48 | | |
| | Low-grade | 11 | 4 | 7 | | | 11 | 2 | 9 | | |
| Depth of invasion | T1 | 8 | 4 | 4 | 2.204 | 0.531 | 8 | 1 | 7 | 2.981 | 0.358 |
| | T2 | 48 | 26 | 22 | | | 48 | 14 | 34 | | |
| | T3 | 31 | 12 | 19 | | | 31 | 10 | 21 | | |
| | T4 | 3 | 2 | 1 | | | 3 | 2 | 1 | | |
| Lymphatic invasion | (-) | 67 | 30 | 37 | 1.975 | 0.383 | 67 | 21 | 46 | 0.225 | 0.635 |
| | (+) | 23 | 14 | 9 | | | 23 | 6 | 17 | | |
| AJCC clinical stage | I+II | 70 | 33 | 37 | 0.384 | 0.535 | 70 | 22 | 48 | 0.306 | 0.580 |
| | III+IV | 20 | 11 | 9 | | | 20 | 5 | 15 | | |

**Table 2.** Correlation between CK1α and β-catenin Ser45-phosphorylation expression in esophageal squamous cell carcinoma

| | | β-catenin Ser45-phosphorylation | | $X^2$ | *P-value* | r |
|---|---|---|---|---|---|---|
| | | + | - | | | |
| CK1α | + | 27 | 17 | 11.397 | 0.001 | 0.356 |
| | - | 12 | 34 | | | |

tween β-catenin Ser45-phosphorylation expression and gender, age, depth of invasion, size of tumors, lymphatic invasion and AJCC clinical stage (**Table 1**). And then Pearson correlation analysis were performed, CK1α and β-catenin Ser45-phosphorylation expression were positively correlated (0.356, P=0.001) (**Table 2**).

## Discussion

Casein kinase 1α (CK1α) is a multifunctional Ser/Thr kinase that phosphorylates several substrates. β-catenin is an important player in Wnt signaling and cell adhesion. Phosphorylation of β-catenin at Ser45 by CKα is the priming phosphorylation, which is the essential reaction for the proteasomal degradation of β-catenin. A down-regulation of CK1α can lead to accumulation of cytoplasmic β-catenin. Interestingly, aside from that, very little is known about the expression, correlation and functional role of CK1α and β-catenin Ser45-phosphorylation in tumor cells.

Recently, only few findings are reported on CK1α, β-catenin Ser45-phosphorylation and tumor. Sinnberg et al found that CK1α is downregulated which correlated with increased β-catenin stability in metastatic melanoma cells, because the phosphorylation of β-catenin by CK1α at Ser45 is suppressed, and CK1α re-expression in metastatic melanoma cells reduces growth in vitro and metastasis formation in vivo, and induces cell cycle arrest and apoptosis [12]. Park et al detected that Calotropin (1) inhibited Wnt signaling by decreasing both nuclear and cytosolic β-catenin nin in a dose-dependent manner, and promoted degradation of β-catenin by increasing the phosphorylation of β-catenin at Ser45 through casein kinase 1α (CK1α), moreover, Calotropin

(1) significantly increased CK1α protein and mRNA levels [13]. Gao's study reported that overexpression of PDIA6 in HeLa cells led to increased cell proliferation accompanied with accelerated cell cycle progression, and further mechanistic investigation demonstrated that overexpression of PDIA6 resulted in decreased phosphorylation of β-catenin at Ser45 and Ser33/Ser37/Thr41, while increased β-catenin nuclear accumulation, and upregulation of Wnt/β-catenin signaling target genes cyclinD1 and c-myc [14]. Cui L showed that pyrvinium inhibits growth and induces apoptosis via caspase pathway in a panel of RCC cell lines, and decreases β-catenin activity and its downstream Wnt-targeted genes transcription via axin-mediated β-catenin protein reduction whereas pyrvinium failed to decrease β-catenin protein level and activity in casein kinase 1α (CK1α)-depleted clear cell RCC cells, demonstrating that pyrvinium inhibits β-catenin in a CK1α-dependent manner [15]. Vaid et al indicated that silymarin, an inhibitor of Wnt/β-catenin pathway, enhanced the levels of casein kinase 1α, glycogen synthase kinase-3β and phosphorylated-β-catenin on critical residues Ser45, Ser33/37 and Thr41, decreased accumulation of nuclear β-catenin and inhibition of MMP-2 and MMP-9 levels, which significantly inhibited cell migration of Mel 1241 cells [16]. Above researches indicated that CK1α and β-catenin Ser45-phosphorylation acted as tumor suppressors by regulating Wnt/β-catenin signaling pathway. However, Bowman's study showed that phosphorylation of FADD by the kinase CK1α promotes the formation of lung cancer in mice [17]. Also β-catenin Ser45-phosphorylation does not necessarily lead to its degradation. The researchers' study found that the APC/phospho-β-catenin complex in cell protrusions appears to be distinct from the APC/axin/β-catenin destruction complex, phosphorylation at Ser45 of β-catenin does not necessarily lead to its degradation but instead regulate cell migration and/or adhesion processes in tumor cells [18]. Iaconelli et al found that HDAC6 inhibitors increased Ser45 phosphorylation, and it resulted in increased membrane localization of β-catenin [19]. These findings suggest that functions of CK1α and β-catenin Ser45-phosphorylation are more complex than originally understood.

In our present study, we examined expression and correlation of CK1α and β-catenin Ser45-phosphorylation in ESCC and nonneoplastic esophageal tissues, and analyzed the clinicopathological data in order to determine the prognosis of the patients. Our study showed that the deregulation of CK1α and phosphorylation of β-catenin at Ser45 in ESCC than the nonneoplastic esophageal tissues, the expression of CK1α and phosphorylation at Ser45 of β-catenin were significantly correlated with the differentiation degree of ESCC. So, the expression of CK1α and phosphorylation at Ser45 of β-catenin may play an important role in the carcinogenesis and tumor progression of ESCC.

In summary, our preliminary research demonstrates that CK1α and phosphorylation of β-catenin at Ser45 provides clinically useful information in cases of ESCC. The expression of CK1α and phosphorylation at Ser45 of β-catenin might be helpful indicators and a potential therapeutic target in ESCC. However, current evidence shows that the role of CK1α and phosphorylation at Ser45 of β-catenin in tumors are still controversial. Therefore, further investigation is still required to explore the molecular mechanisms of CK1α and phosphorylation at Ser45 of β-catenin in ESCC.

## Acknowledgements

This research was supported by the science and technology innovation team program of Changzhi Medical College (CX201407), and Shanxi Province Service Industry Innovation Discipline Group Construction Plan (NO. 20-1809).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Jinsheng Wang, Department of Pathology, Changzhi Medical College, Changzhi, Shanxi, China. Tel: +86-1563559-7999; E-mail: tigergv@163.com

## References

[1] Pennathur A, Gibson MK, Jobe BA and Luketich JD. Oesophageal carcinoma. Lancet 2013; 381: 400-412.

[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

[3] Ke L. Mortality and incidence trends from esophagus cancer in selected geographic ar-

eas of China circa 1970-90. Int J Cancer 2002; 102: 271-274.
[4] McKay RM, Peters JM and Graff JM. The casein kinase I family in Wnt signaling. Dev Biol 2001; 235: 388-396.
[5] Price MA. CKI, there is more than one: casein kinase I family members in Wnt and hedgehog signaling. Genes Dev 2006; 20: 399-410.
[6] Knippschild U, Krüger M, Richter J, Xu P, García-Reyes B, Peifer C, Halekotte J, Bakulev V and Bischof J. The CK1 family: contribution to cellular stress response and its role in carcinogenesis. Front Oncol 2014; 4: 96.
[7] Schittek B and Sinnberg T. Biological functions of casein kinase 1 isoforms and putative roles in tumorigenesis. Mol Cancer 2014; 13: 231.
[8] Cruciat CM. Casein kinase 1 and Wnt/β-catenin signaling. Curr Opin Cell Biol 2014; 31: 4655.
[9] Bernatik O, Ganji RS, Dijksterhuis JP, Konik P, Cervenka I, Polonio T, Krejci P, Schulte G and Bryja V. Sequential activation and inactivation of dishevelled in the Wnt/beta-catenin pathway by casein kinases. J Biol Chem 2011; 286: 10396-410.
[10] Edge SB, Byrd DR, Compton CC, et al. AJCC cancer staging manual 7th edition. New York: Springer; 2010.
[11] Ren HZ, Wang JS, Wang P, Pan GQ, Wen JF, Fu H and Shan XZ. Increased expression of prohibitin and its relationship with poor prognosis in esophageal squamous cell carcinoma. Pathol Oncol Res 2010; 16: 515-522.
[12] Sinnberg T, Menzel M, Kaesler S, Biedermann T, Sauer B, Nahnsen S, Schwarz M, Garbe C and Schittek B. Suppression of casein kinase 1alpha in melanoma cells induces a switch in beta-catenin signaling to promote metastasis. Cancer Res 2010; 70: 6999-7009.
[13] Park HY, Toume K, Arai MA, Sadhu SK, Ahmed F and Ishibash M. Calotropin: a cardenolide from calotropis gigantea that inhibits Wnt signaling by increasing casein kinase 1α in colon cancer cells. Chembiochem 2014; 15: 872-8.
[14] Gao H, Sun B, Fu H, Chi X, Wang F, Qi X, Hu J and Shao S. PDIA6 promotes the proliferation of HeLa cells through activating the Wnt/β-catenin signaling pathway. Oncotarget 2016; 7: 53289-53298.
[15] Cui L, Zhao J and Liu J. Pyrvinium sensitizes clear cell renal cell carcinoma response to chemotherapy via casein kinase 1α-dependent inhibition of Wnt/β-Catenin. Am J Med Sci 2018; 355: 274-280.
[16] Vaid M, Prasad R, Sun Q and Katiyar SK. Silymarin targets β-catenin signaling in blocking migration/invasion of human melanoma cells. PLoS One 2011; 6: e23000.
[17] Bowman BM, Sebolt KA, Hoff BA, Boes JL, Daniels DL, Heist KA, Galbán CJ, Patel RM, Zhang J, Beer DG, Ross BD, Rehemtulla A and Galbán S. Phosphorylation of FADD by the kinase CK1α promotes KRASG12D-induced lung cancer. Sci Signal 2015; 27: 361.
[18] Faux MC, Coates JL, Kershaw NJ, Layton MJ and Burgess AW. Independent interactions of phosphorylated β-catenin with E-cadherin at cell-cell contacts and APC at cell protrusions. PLoS One 2010; 5: e14127.
[19] Iaconelli J, Huang JH and Berkovitch SS. HDAC6 inhibitors modulate Lys49 acetylation and membrane localization of beta-catenin in human iPSC-derived neuronal cells. ACS Chem Biol 2015; 10: 883-890.