College of Animal Science and Veterinary Medicine[1], Shanxi Agricultural University, Taigu; Beijing University of Chinese Medicine[2], Beijing, China; Transplantation Biology Research Center[c], Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Oleanolic acid derivatives inhibit the Wnt/β-catenin signaling pathway by promoting the phosphorylation of β-catenin in human SMMC-7721 cells

Xinfeng Fan [1,*], Penglong Wang [2,*], Yaogui Sun[1], Junbing Jiang[1], Haiyuan Du[1], Zhirui Wang[3], Zhibian Duan[1], Haimin Lei[2], Hongquan Li[1]

Received February 6, 2016, accepted March 11, 2016

Haimin Lei, Beijing University of Chinese Medicine, Beijing 100000, China
leihaimin@126.com
Hongquan Li, College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu 030801, China
livets@163.com
**These authors contributed to the work equally and should be regarded as co-first authors.*

Pharmazie 71: 398–401 (2016) *doi: 10.1691/ph.2016.6536*

Oleanolic acid, isolated from *privet*, has shown antitumor effects in several cancers. However, the underlying molecular mechanism associated with these effects is largely unknown. In this study, we explored the effect of oleanolic acid derivatives on the Wnt/β-catenin signaling pathway in human hepatocellular carcinoma SMMC-7721 cells. The mRNA and protein levels of related genes were determined by real-time quantitative PCR and Western blot, respectively. Treatment of SMMC-7721 cells with oleanolic acid derivatives led to the downregulation of the mRNA and protein levels of β-catenin, c-myc, and cyclin D1. Treatment with oleanolic acid derivatives decreased the levels of β-catenin in both the cytoplasm and the nucleus. Moreover, oleanolic acid derivatives promoted the phosphorylation of β-catenin (Ser33/37/Thr41) in the cytoplasm. Our results suggest that oleanolic acid derivatives inhibit the Wnt/β-catenin signaling pathway by stimulating the phosphorylation of β-catenin (Ser33/37/Thr41) in human SMMC-7721 cells.

1. Introduction

Hepatocellular carcinoma (HCC) has a high mortality and incidence rate (Venook et al. 2010). HCC accounts for approximately one million deaths per year worldwide. Surgical removal of liver cancer tissue and organ transplantation therapies remain the predominant first-line therapeutic measures for HCC. Radiofrequency ablation and percutaneous ethanol ablation are widely used to control the disease when surgery and organ transplantation is not achievable. Chemotherapy and immunological and hormonal therapies constitute alternative treatment regimens. However, these treatments can cause liver cirrhosis and liver damage. In recent years, molecular targeted drugs such as sorafenib have been utilized to treat liver cancer. These drugs have improved the survival rate of liver cancer patients; however, eventual drug resistance is inevitable.

To facilitate the development of drugs to treat liver cancer, it is essential to elucidate the molecular mechanisms of the initiation, progression, and survival of liver cancer cells. Different cancer cell types have facilitated abnormal activation of distinct signaling pathways including Wnt, and Ras/extracellular signal-regulated kinase (RAS/ERK) (Tanaka and Arii 2012). Aberrant activation of the Wnt signaling cascade is closely associated with the occurrence of various cancers (Mazieres et al. 2005; Tennis et al. 2007), most notably HCC (Kaur et al. 2012). In animal experiments, transgenetic upregulation of Wnt signaling has been shown to cause tumor development (Cadigan and Nusse 1997; Clevers 2006). Clinical studies have demonstrated that the Wnt pathway is aberrant in nearly a third of patients with HCCs (Boyault et al. 2007; Chiang et al. 2008). Two different Wnt signaling cascades have been identified: non-canonical and canonical pathways. The Wnt/β-catenin signaling pathway plays an important role in the regulation of cell proliferation and differentiation, and is deregulated in many cancers and other diseases (Takahashi-Yanaga and Sasaguri 2007). Thus, targeting Wnt/β-catenin pathways is a promising strategy for the development of drugs for the treatment of HCC.

Oleanolic acid (OA) and ursolic acid (UA) are isomers. OA, UA, and their associated derivatives belong to a group of compounds known as the pentacyclic triterpene compounds and are abundant in various plants. In recent years, OA, UA, and their derivatives have been shown to induce cancer cell apoptosis through various pathways in many different kinds of cancer cells, including human pancreatic cancer cells, colonic cancer cells, breast cancer cells, bladder cancer cells, ovarian cancer cells, and hepatocellular carcinoma cells (Chakravarti et al. 2012; Shan et al. 2011; Shyu et al. 2010; Song et al. 2012; Wei et al. 2013; Zheng et al. 2013). It is reported that OA causes cell cycle arrest and induces apoptosis in pancreatic cancer cells through mitochondrial depolarization (Wei et al. 2013). UA has been shown to induce cell apoptosis in SW480 cells by lowering the expression level of the apoptosis antagonistic proteins, for example Bcl-2, Bcl-xL (Shan et al. 2011). Moreover, UA induces human bladder cancer cellular apoptosis by activating apoptosis signal-regulating kinase 1- Jun N-terminal kinase (ASK1-JNK) signaling (Zheng et al. 2013). In addition, UA induces SK-OV-3 ovarian cancer cells apoptosis through activation of the phosphorylation of GSK-3β (Song et al. 2012). Furthermore, OA and UA induce HuH7 cells apoptosis by downregulating X linked inhibitor of apoptosis protein (XIAP) (Shyu et al. 2010). These data suggest that OA, UA, and their derivatives inhibit the growth of many kinds of cancer cells by targeting distinct signaling pathways.

In recent years, active components from traditional Chinese medicine have attracted a great deal of attention as candidates for HCC therapy. Numerous phytochemicals have been identified to disrupt Wnt/β-catenin signaling, such as lupeol, flavanoids (genistein), lycopene, curcumin, epigallocatechin-3-gallate (EGCG), and resveratrol (Tarapore et al. 2012). However, it remains unclear whether OA, UA, or their derivatives alter the activity of Wnt/β-catenin signaling in HCC. In the present study, the effect of OA derivatives on the Wnt/β-catenin signaling pathway in HCC was explored.

2. Investigations and results

2.1. Oleanolic acid derivatives downregulate the mRNA and protein expression of β-catenin, c-myc, and cyclin D1

In order to test whether oleanolic acid derivatives affect the activity of the Wnt/β-catenin signal transduction pathway, SMMC-7721 cells were treated with oleanolic acid derivatives (0, 7, 9, and 11 μM) for 48 h. mRNA and protein levels of β-catenin, C-myc and Cyclin D1 were detected using Real-time qPCR and immunoblotting, respectively. The results demonstrated that oleanolic acid derivatives reduced the mRNA level of β-catenin with a significant reduction following treatment with 11 μM of the derivatives (Fig. 1A). Moreover, the mRNA expression levels of C-myc (Fig. 1B) and cyclin D1 (Fig. 1C) were significantly downregulated by incubation with oleanolic acid derivatives in a dose-dependent manner. In addition, the oleanolic acid derivatives dose-dependently downregulated the protein levels of β-catenin, C-myc and Cyclin D1 (Fig. 1D). These results indicated that the oleanolic acid derivatives inhibited the Wnt/β-catenin signaling pathway in SMMC-7721 cells.

2.2. Oleanolic acid derivative induces the phosphorylation of cytoplasmic β-catenin

β-Catenin is the key molecule of the Wnt-signaling pathway. After dissociation from membrane-associated adheren junctions into the cytoplasm, β-catenin is ubiquitinated for degradation when phosphorylated at Ser33/Ser37/Thr41. After activation of the Wnt-signaling pathway, β-catenin (Ser33/Ser37/Thr41) phosphorylation is generally inhibited, leading to its stabilization. The stabilized form of β-catenin subsequently enters the nucleus to regulate gene expression (Behrens et al. 1998). To test the effects of the oleanolic acid derivatives on the intracellular translocation of β-catenin, we checked the cytoplasmic and nuclear protein levels of β-catenin following immunoblotting. Total β-catenin was decreased in both the cytoplasm fraction (Fig. 2A) and the nuclear fraction (Fig. 2B) in a concentration-dependent manner following treatment with the oleanolic acid derivatives. Intriguingly, phosphorylation of β-catenin (Ser33/37/Thr41) derivatives in the cytoplasm fraction was dramatically increased following incubation with the oleanolic acid (Fig. 2A). These results indicate that the oleanolic acid derivatives increased the phosphorylation of cytoplasmic β-catenin in SMMC-7721 cells.

2.3. Oleanolic acid derivatives increase the phosphorylation level of total *β*-catenin

To confirm that the enhanced phosphorylation levels of total β-catenin were not due to the alteration of the total β-catenin, we detected the total β-catenin after cells were treated with oleanolic acid derivatives for 24 h. We observed that oleanolic acid derivatives treatment of SMMC-7721 cells for 24 h did not alter the amount of β-catenin

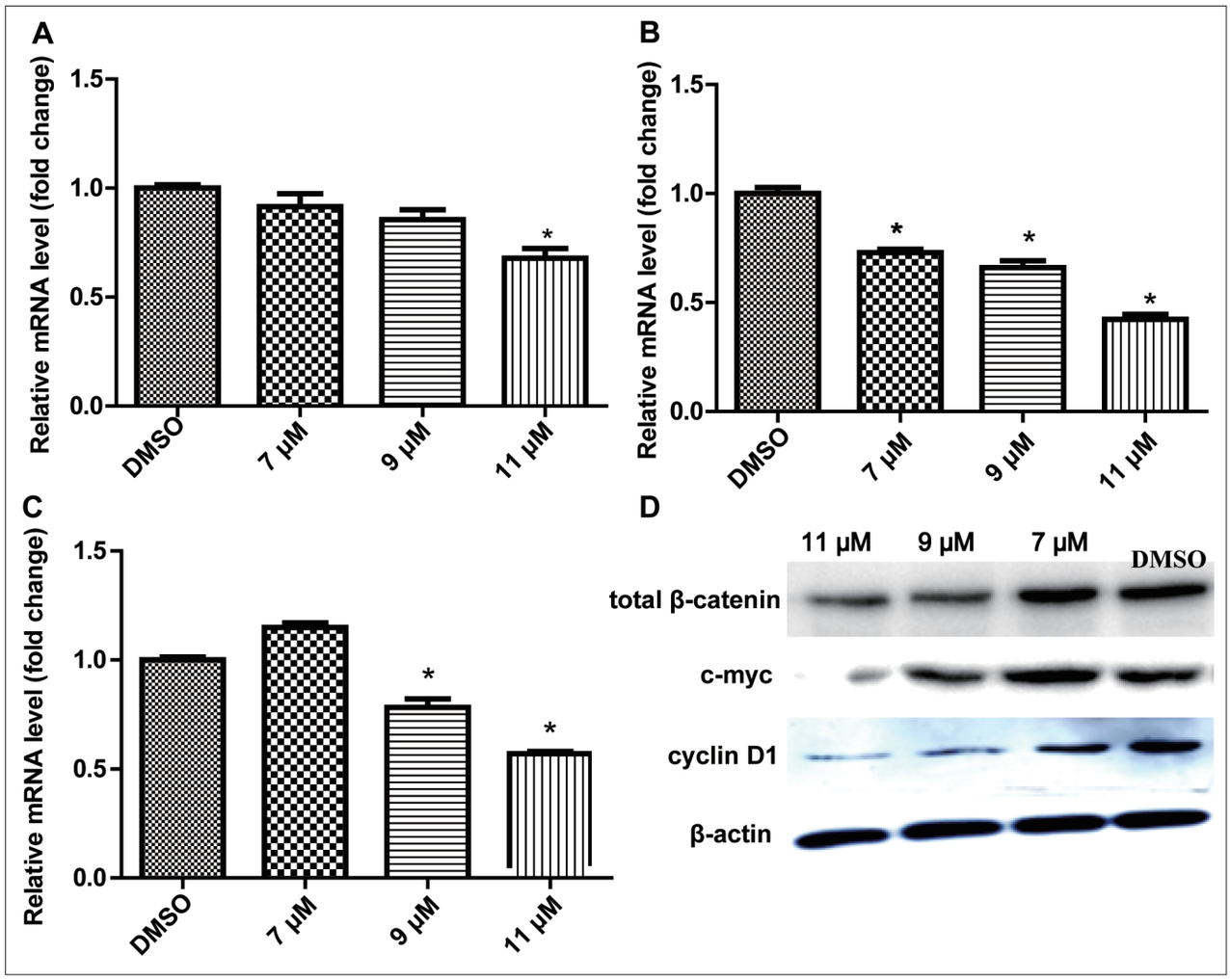

Fig. 1: Oleanolic acid derivatives suppress the activation of Wnt/β-catenin signaling in SMMC-7721 cells. SMMC-7721 cells were incubated with 7, 9, or 11 μM of oleanolic acid derivatives for 48 h. RT-PCR was used to determine the mRNA levels of β-catenin (A), c-myc (B) and cyclin D1 (C) with b-actin used as an internal control. (D) SMMC-7721 cells were treated with 7, 9, or 11 μM oleanolic acid derivatives for 48 h. The extracted total protein was analyzed for β-catenin, c-myc, and cyclinD1 with b-actin used as loading control. All values are mean ± SD of three experiments. * $p<0.05$ vs. DMSO.

protein. However, the phosphorylation of β-catenin (Ser33/37/Thr41) from the whole cell extracts was obviously increased by oleanolic acid derivatives in a concentration-dependent manner (Fig. 3). The above results show that oleanolic acid derivatives promote the phosphorylation of β-catenin, thereby leading to its degradation.

3. Discussion

In this study, we found that oleanolic acid derivatives reduced the mRNA and protein expression levels of β-catenin, c-myc, cyclin D1. Moreover, oleanolic acid derivatives increased the levels of phospho-β-catenin (Ser33/37/Thr41). Our results suggest that oleanolic acid derivatives inhibit the activity of the Wnt/β-catenin pathway through enhancing the phosphorylation of β-catenin (Ser33/37/Thr41), thereby promoting its degradation in human SMMC-7721 cells.

Tumorigenesis is closely related to the Wnt/β-catenin signal transduction pathway (Takahashi-Yanaga and Kahn 2010). It has been shown that upregulation of the Wnt/β-catenin pathway promotes the proliferation, migration and invasion of HCC cells (Pez et al. 2013). Moreover, deregulation of β-catenin is closely related with the tumorigenesis of HCC (Zeng et al. 2007). Cytoplasmic β-catenin is normally kept at a low level through the ubiquitin-proteasome system, which is regulated by a "destruction" complex made up of axin, APC and GSK-3β. Binding of Wnt proteins to Frizzled receptors leads to an increase in the levels of non-phosphorylated β-catenin, which enter into the nucleus, binds with the T-cell transcription factor/lymphoid enhancer-binding factor (TCF/LEF) and activates the expression of c-Myc and cyclin D1 (Clevers 2006; Moon et al. 2004). In different cancers, c-Myc is frequently overexpressed (Lin C. P. et al. 2010). Much evidence suggests that c-myc is a potential drug target (Calcagno et al. 2008; Dang 2012; Li et al. 2003; Wang et al. 2012). Leu et al. (2014) reported that reevesioside A can inhibit c-myc protein expression to exert its anticancer activity against human hormone-refractory prostate cancers. The development and progression of many cancers such as breast, bladder and lung cancers depend on cyclin D1. Many studies found that the degradation of cyclin D1 may enhance the therapeutic intervention (Alao et al. 2006; Alao et al. 2004; Feng et al. 2007). In the present study, we found that oleanolic acid derivatives decreased the expression of β-catenin, c-Myc, cyclin D1 in a dose-dependent manner in SMMC-7721 cells.

It is well known that non-phosphorylated β-catenin avoids ubiquitin-proteasome-mediated degradation and subsequently translocates into the nucleus. High levels of nuclear β-catenin are responsible for poor prognosis in some cancers (Baldus et al. 2004; Cheng et al. 2011; Khramtsov et al. 2010; Lin et al. 2000). In the present study, we found that oleanolic acid derivatives reduced the expression of β-catenin regardless of the cytoplasm and nucleus in a concentration -dependent manner. GSK-3β phosphorylates β-catenin (Ser33/37/Thr41) and facilitates the maintenance of low levels of β-catenin in the cytoplasm. We hypothesized that the reduction in cytoplasmic β-catenin following oleanolic acid derivative treatment might be due to the upregulation of phospho-β-catenin (Ser33/37/Thr41), thereby promoting its degradation. Indeed, the results indicated that oleanolic acid derivatives enhanced the phosphorylation of β-catenin in the cytoplasm. Evidence has indicated that β-catenin is important in hepatocarcinogenesis and assists in maintaining the survival of HCC cells, making it a potential target for cancer therapeutics (Zeng et al. 2007). It is therefore important that the mechanism by which oleanolic acid derivatives induce the phosphorylation of β-catenin will be further studied.

In conclusion, oleanolic acid derivatives suppress the Wnt/β-catenin signaling pathway in HCC cells. This is accompanied by increased phosphorylation of cytosolic β-catenin. Our findings suggest that one of the mechanisms associated with the antitumor activity of OA, UA and their derivatives might be through blocking the Wnt/β-catenin pathway.

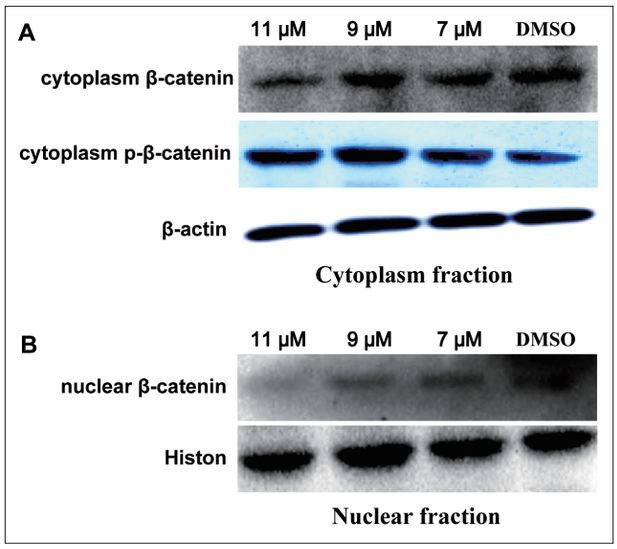

Fig. 2: Oleanolic acid derivatives induced the phosphorylation of β-catenin. (A) SMMC-7721 cells were incubated with 7, 9, or 11 μM of oleanolic acid derivatives for 48 h. Cytoplasmic proteins were extracted for Western blot analysis of β-catenin and phosphorylation of β-catenin (Ser33/37/Thr41) with β-actin as a reference. (B) Nuclear proteins were extracted for analysis of β-catenin with Histone H2A as loading control. DMSO was used as a control treatment.

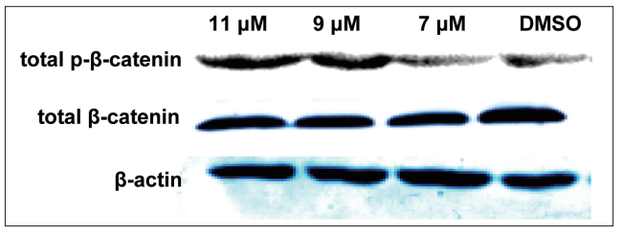

Fig. 3: Oleanolic acid derivative promoted the phosphorylation of total β-catenin. Total protein from SMMC-7721 cells treated with 0, 7, 9, or 11 μM of oleanolic acid derivatives for 24 h was analyzed for β-catenin and phosphorylation of β-catenin (Ser33/37/Thr41) with β-actin as a loading control.

4. Experimental

4.1. Chemicals and reagents

Oleanolic acid derivatives were kindly given by Dr. Lei of Beijing University of Chinese Medicine (Beijing, China). The details of the oleanolic acid derivatives have been introduced in previous studies. The derivatives of 93% purity were subsequently dissolved in dimethylsulfoxide (DMSO). RPMI1640 cell culture medium and fetal bovine serum (FBS) were purchased from Gibco. Sodium dodecylsulfate (SDS), dithiothreitol (DTT), phenylmethylsulfonylfluoride (PMSF), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), annexin V-FITC/propidium iodide (PI) apoptosis detection kit and JC-1 were purchased from Sigma. Reverse transcriptase and the SYBR green quantitative RT-PCR kit were purchased from TAKARA (Dalian, China). Rabbit anti-β-catenin, anti-cyclin D1, anti-c-myc, anti-β-actin, phospho-β-Catenin (Ser33/37/Thr41) and horseradish epoxidase-conjugated goat anti-rabbit antibodies were obtained from Cell Signaling Technology (Boston, MA, USA). The nucleoprotein and cytoplasm protein extraction kits were obtained from Nanjing KeyGen Biotech (Nanjing, China). PVDF microporous membranes and the enhanced chemiluminescence detection kit were obtained from TAKARA (Dalian, China). The RIPA cell lysis buffer was obtained from Beyotime Biotech (Haimen, China).

4.2. Cell culture and treatment

Human HCC SMMC-7721 cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and were cultivated in RPMI-1640 medium containing 10% heat inactivated FBS and 1% penicillin-streptomycin at 37 °C in a 5% CO_2 incubator. Cells were treated with different concentrations of oleanolic acid derivatives or DMSO (alone) for the indicated period of time.

4.3. The genetic level analysis test

Cells were maintained in 6-well plates and treated with 7, 9, or 11 μM of oleanolic acid derivatives or 0.11% DMSO for 48 h. Trizol agent (Gibco Life Technologies) was used for extraction of general RNA and RNA concentration was determined using a spectrophotometer (Thermo-Scientific, Wilmington, DE). The quality of RNA was visualized using 1% agarose gel electrophoresis. RT-PCR was completed in a 20 μl reaction mixture using the following procedure: predegeneration (95 °C, 30 s), and 35 cycles of denaturation (94 °C, 45 s), annealing (60 °C, 30 s) and extension (72 °C, 1 min). The terminal step was an

Table: Sequences of primers for RT-PCR

Gene	Sense (5'-3')	Antisense (5'-3')
β-catenin	CCAAGTGGGTGGTATAGAGG	AGTCCATAGTGAAGGCGAAC
c-myc	TTGTTGCGGAAACGACG	TCATAGGTGATTGCTCAGGAC
cyclinD1	GCATGTTCGTGGCCTCTAAG	TTCAATGAAATCGTGCGGGG
β-actin	AAATCGTGCGTGACATTAA	GGAAGGAAGGTTGGAAGAGAGC

extension step at 72 °C for 10 min. The threshold cycle (Ct) has an inverse association with the related mRNA level. The 2-ΔΔCt method was used to calculate the relative quantity of target mRNA. The sequences of the primers were designed using Primer Premier 5.0 and are shown in the Table.

4.4. Western blot analysis

Cell lysis buffer containing a 1% protease inhibitor cocktail (Roche) was used to extract the total protein. Cytoplasm and nuclear proteins were prepared using a cytoplasmic and nuclear protein extraction kit according to manufacturer's instructions. Protein concentration was determined using the BCA assay. A total of 60 μg of extracted protein was separated on 10% SDS-PAGE and subsequently transferred onto a PVDF membrane. The membranes were blocked with 5% w/v non-fat dry milk dissolved in TBS-T at room temperature for 1 h and incubated with primary antibodies overnight at 4 °C. Twelve hours later, PVDF membranes were washed with TBS-T, and incubated with horseradish peroxidase (HRP)-labeled secondary antibodies for 60 min at room temperature. An ECL kit was used for observing protein bands. β-actin acted as a loading control.

4.5. Statistical analysis

Data are presented as mean±SD. All experiments were repeated at least three times. All data were analyzed using SPSS19.0 statistical software. One-way ANOVA was used to analyze multiple groups. GraphPad Prism 5.02 and Excel were used for statistical analysis. A P value < 0.05 was considered significantly different.

Acknowledgments: This research was funded by the Agricultural science and technology achievements transformation projects (2014GB2A300003).

Conflict of interest: The authors declare no conflicts of interest.

References

Alao JP, Stavropoulou AV, Lam EW, Coombes RC, Vigushin DM (2006) Histone deacetylase inhibitor, trichostatin A induces ubiquitin-dependent cyclin D1 degradation in MCF-7 breast cancer cells. Mol Cancer 5: 8.

Alao JP, Lam EW, Ali S, Buluwela L, Bordogna W, Lockey P, Varshochi R, Stavropoulou AV, Coombes RC, Vigushin DM (2004) Histone deacetylase inhibitor trichostatin A represses estrogen receptor alpha-dependent transcription and promotes proteasomal degradation of cyclin D1 in human breast carcinoma cell lines. Clin Cancer Res 10: 8094-8104.

Baldus SE, Monig SP, Huxel S, Landsberg S, Hanisch FG, Engelmann K, Schneider PM, Thiele J, Hölscher AH, Dienes HP (2004) MUC1 and nuclear β-catenin are coexpressed at the invasion front of colorectal carcinomas and are both correlated with tumor prognosis. Clin Cancer Res 10: 2790-2796.

Behrens J, Jerchow BA, Wurtele M, Grimm J, Asbrand C, Wirtz R, Kühl M, Wedlich D, Birchmeier W (1998) Functional interaction of an axin homolog, conductin, with β-catenin, APC, and GSK3β. Science 280: 596-599.

Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, Jeannot E, Hérault A, Saric J, Belghiti J, Franco D, Bioulac-Sage P, Laurent-Puig P, Zucman-Rossi J (2007) Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology 45: 42-52.

Cadigan KM, Nusse R (1997) Wnt signaling: a common theme in animal development. Genes Dev 11: 3286-3305.

Calcagno DQ, Leal MF, Assumpcao PP, Smith MA, Burbano RR (2008) MYC and gastric adenocarcinoma carcinogenesis. World J Gastroenterol 14: 5962-5968.

Chakravarti B, Maurya R, Siddiqui JA, Bid HK, Rajendran SM, Yadav PP, Konwar R (2012) In vitro anti-breast cancer activity of ethanolic extract of Wrightia tomentosa: role of pro-apoptotic effects of oleanolic acid and urosolic acid. J Ethnopharmacol 142: 72-79.

Cheng H, Liang H, Qin Y, Liu Y (2011) Nuclear β-catenin overexpression in metastatic sentinel lymph node is associated with synchronous liver metastasis in colorectal cancer. Diagn Pathol 6: 109.

Chiang DY, Villanueva A, Hoshida Y, Peix J, Newell P, Minguez B, LeBlanc AC, Donovan DJ, Thung SN, Solé M, Tovar V, Alsinet C, Ramos AH, Barretina J, Roayaie S, Schwartz M, Waxman S, Bruix J, Mazzaferro V, Ligon AH, Najfeld V, Friedman SL, Sellers WR, Meyerson M, Llovet JM (2008) Focal gains of VEGFA and molecular classification of hepatocellular carcinoma. Cancer Res 68: 6779-6788.

Clevers H (2006) Wnt/β-catenin signaling in development and disease. Cell 127: 469-480.

Dang CV (2012) MYC on the path to cancer. Cell 149: 22-35.

Feng Q, Sekula D, Muller R, Freemantle SJ, Dmitrovsky E (2007) Uncovering residues that regulate cyclin D1 proteasomal degradation. Oncogene 26: 5098-5106.

Kaur P, Mani S, Cros MP, Scoazec JY, Chemin I, Hainaut P, Herceg Z (2012) Epigenetic silencing of sFRP1 activates the canonical Wnt pathway and contributes to increased cell growth and proliferation in hepatocellular carcinoma. Tumour Biol 33: 325-336.

Khramtsov AI, Khramtsova GF, Tretiakova M, Huo D, Olopade OI, Goss KH (2010) Wnt/β-catenin pathway activation is enriched in basal-like breast cancers and predicts poor outcome. Am J Pathol 176: 2911-2920.

Leu WJ, Chang HS, Chan SH, Hsu JL, Yu CC, Hsu LC, Chen IS, Guh JH (2014) Reevesioside A, a cardenolide glycoside, induces anticancer activity against human hormone-refractory prostate cancers through suppression of c-myc expression and induction of G1 arrest of the cell cycle. PLoS One 9: e87323.

Li Z, Van Calcar S, Qu C, Cavenee WK, Zhang MQ, Ren B (2003) A global transcriptional regulatory role for c-Myc in Burkitt's lymphoma cells. Proc Natl Acad Sci U S A 100: 8164-8169.

Lin CP, Liu CR, Lee CN, Chan TS, Liu HE (2010) Targeting c-Myc as a novel approach for hepatocellular carcinoma. World J Hepatol 2: 16-20.

Lin SY, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y, Pestell RG, Hung MC (2000) β-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. Proc Natl Acad Sci U S A 97: 4262-4266.

Mazieres J, He B, You L, Xu Z, Jablons DM (2005) Wnt signaling in lung cancer. Cancer Lett 222: 1-10.

Moon RT, Kohn AD, De Ferrari GV, Kaykas A (2004) WNT and β-catenin signalling: diseases and therapies. Nat Rev Genet 5: 691-701.

Pez F, Lopez A, Kim M, Wands JR, Caron de Fromentel C, Merle P (2013) Wnt signaling and hepatocarcinogenesis: molecular targets for the development of innovative anticancer drugs. J Hepatol 59: 1107-1117.

Shan JZ, Xuan YY, Ruan SQ, Sun M (2011) Proliferation-inhibiting and apoptosis-inducing effects of ursolic acid and oleanolic acid on multi-drug resistance cancer cells *in vitro*. Chin J Integr Med 17: 607-611.

Shyu MH, Kao TC, Yen GC (2010) Oleanolic acid and ursolic acid induce apoptosis in HuH7 human hepatocellular carcinoma cells through a mitochondrial-dependent pathway and downregulation of XIAP. J Agric Food Chem 58: 6110-6118.

Song YH, Jeong SJ, Kwon HY, Kim B, Kim SH, Yoo DY (2012) Ursolic acid from Oldenlandia diffusa induces apoptosis via activation of caspases and phosphorylation of glycogen synthase kinase 3 beta in SK-OV-3 ovarian cancer cells. Biol Pharm Bull 35: 1022-1028.

Takahashi-Yanaga F, Sasaguri T (2007) The Wnt/β-catenin signaling pathway as a target in drug discovery. J Pharmacol Sci 104: 293-302.

Takahashi-Yanaga F, Kahn M (2010) Targeting Wnt signaling: can we safely eradicate cancer stem cells? Clin Cancer Res 16: 3153-3162.

Tanaka S, Arii S (2012) Molecular targeted therapies in hepatocellular carcinoma. Semin Oncol 39: 486-492.

Tarapore RS, Siddiqui IA, Mukhtar H (2012) Modulation of Wnt/β-catenin signaling pathway by bioactive food components. Carcinogenesis 33: 483-491.

Tennis M, Van Scoyk M, Winn RA (2007) Role of the wnt signaling pathway and lung cancer. J Thorac Oncol 2: 889-892.

Venook AP, Papandreou C, Furuse J, de Guevara LL (2010) The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. Oncologist 15 Suppl 4: 5-13.

Wang J, Kobayashi T, Floc'h N, Kinkade CW, Aytes A, Dankort D, Lefebvre C, Mitrofanova A, Cardiff RD, McMahon M, Califano A, Shen MM, Abate-Shen C (2012) B-Raf activation cooperates with PTEN loss to drive c-Myc expression in advanced prostate cancer. Cancer Res 72: 4765-4776.

Wei J, Liu M, Liu H, Wang H, Wang F, Zhang Y, Han L, Lin X (2013) Oleanolic acid arrests cell cycle and induces apoptosis via ROS-mediated mitochondrial depolarization and lysosomal membrane permeabilization in human pancreatic cancer cells. J Appl Toxicol 33: 756-765.

Zeng G, Apte U, Cieply B, Singh S, Monga SP (2007) siRNA-mediated β-catenin knockdown in human hepatoma cells results in decreased growth and survival. Neoplasia 9: 951-959.

Zheng QY, Li PP, Jin FS, Yao C, Zhang GH, Zang T, Ai X (2013) Ursolic acid induces ER stress response to activate ASK1-JNK signaling and induce apoptosis in human bladder cancer T24 cells. Cell Signal 25: 206-213.