# Curcumin suppresses proliferation and induces apoptosis of human hepatocellular carcinoma cells via the wnt signaling pathway

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**Abstract.** Curcumin from the rhizome of *Curcuma longa* (*zingiberaceae*) has been reported to be a chemopreventive agent that affects cell proliferation by arresting the cell cycle in G2 and modulating the wnt signaling pathway. We found that curcumin inhibits proliferation and induces apoptosis of human hepatocellular carcinoma (HCC) cells in a concentration-dependent manner. We identified that curcumin interrupts wnt signaling by decreasing β-catenin activity, which in turn suppresses the expression of β-catenin target genes (c-myc, VEGF and cyclin D1). Our results from molecular simulation of curcumin binding to Dvl2 protein and from binding free energy calculations suggest that curcumin may prevent axin recruitment to cellular membrane in order to maintain the functional β-catenin destruction complex in normal cells. This results in β-catenin being unable to accumulate in the nucleus, depriving the protein of its ability to bind with lymphoid enhancer factor/T cell-specific transcription factor (Lef/Tcf) and repressing its activation of target gene transcription. This may be one mechanism through which curcumin inhibits proliferation and induces apoptosis of HCC cells.

## Introduction

Wnts are secreted cysteine-rich glycoproteins that convey signals from donor cells to adjacent recipient cells in a paracrine fashion (1). Wnt homologues emerged from the simplest multicellular organisms, suggesting that wnt signaling pathway is one of the most fundamental signaling pathways in orchestrating cell-cell communication, tissue development and organogenesis (2). The earliest studies in identifying wnt homologues and their functions were on *C. elegans* and *Drosophila* (3). Two decades after these pioneering explorations, 19 structurally conserved wnt family members have been identified in humans (4), from which the vastly complicated wnt regulation network was gradually established. Current knowledge shows that wnt signaling pathway plays important roles in embryonic development, postnatal tissue homeostasis and pathogenesis (5).

The most studied wnt downstream signaling is through to be the β-catenin dependent pathway, referred to as wnt canonical signaling pathway (6,7). The core issue of the wnt canonical signaling pathway is the competition between elimination and accumulation of its major downstream mediator, β-catenin. Free β-catenin can be translocated and accumulated to the nucleus, where it binds to lymphoid enhancer factor/T cell-specific transcription factor (Lef/Tcf) in order to activate the transcription of target genes (8). Although cells consistently synthesize β-catenin to maintain a potent responsiveness for incoming wnt signaling, the high turnover rate of β-catenin significantly limits β-catenin nuclear accumulation and the target gene expression (9).

Many studies found that various types of cancer including colorectal, pancreatic, lung and prostate cancer, and leukemia are dependent on wnt aberrant signaling (10-12). In fact, more than 80% colorectal cancers and 90% of renal cell carcinoma (RCC, the most frequent type of kidney cancer) are related to mutations in the wnt signaling pathway (13-17). Hence, targeting the wnt signaling could be a promising strategy in treating these diseases (18). However, systematic manipulation of wnt signaling may prove beneficial in treating certain diseases, it may cause damage to normal tissues that depend on the pathway (19,20).

Curcumin is a natural phenol extract from *zingiberaceae*. Preclinical studies suggest that curcumin has potential functions in preventing and treating many diseases, such as colon, gastric, intestinal and breast cancers, inflammatory diseases and HIV (21,22). It was reported that the chemo-preventive capability of curcumin was exactly through the modulation of the wnt canonical signaling pathway (23).

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The purpose of this study was to gain insight into the molecular mechanism of curcumin involved in the effects of wnt/β-catenin signaling pathway. Docking simulations *in silico*, binding free energy calculations, MTT assay, FACS-based apoptosis assay, immunoblot and RT-PCR were among the techniques used to validate our findings.

## Materials and methods

*Molecular docking between curcumin and Dvl2-PDZ domain*. The molecular 3-D structure of Dvl2-PDZ domain was extracted from the Protein Data Bank (www.pdb.org, PDB CODE: 3CBY). The chemical structure of curcumin was constructed and optimized in the implicit solvent as preparation for docking, and the docking performance was executed employing the CDOCKER module on the platform of Discovery studio 2.5 (Accelrys Inc.). The critical peptide ligand binding site was defined as docking region (24). The conformation search space was limited to a spherical region with the center of (37.6, 3.0, 7.3) and the radius of 9.5 Å. The other parameters were set according to the default values of the module, with all docking procedures using CHARMm forcefield, and a grid extension of 8.0. The ligand partial charge method was also used according to CHARMm. Ten top hits were obtained as docking results. Simulated annealing method was employed for the final conformation treatment: the system was heated to 700 K with 2,000 steps and then cooled to 300 K with 5,000 steps. Finally, the best conformation was selected as the object of analysis according to the values of score.

*Binding free energy calculation*. Binding free energy calculation was performed with MM-PBSA module of Amber12 (25). In the MM-PBSA method, the free energy of the receptor/ligand binding, $\Delta G_{bind}$, is obtained from the difference between the free energies of the receptor/ ligand complex ($\Delta G_{cpx}$) and the unbound receptor ($\Delta G_{rec}$) and ligand ($\Delta G_{lig}$) as following:

$$\Delta G_{bind} = \Delta G_{cpx} - (\Delta G_{rec} + \Delta G_{lig}) \quad (1)$$

The binding free energy ($\Delta G_{bind}$) is evaluated as a sum of the changes of molecular mechanical (MM) gas-phase binding energy ($\Delta E_{MM}$), solvation free energy ($\Delta G_{sol}$) and entropic contribution (-T$\Delta$S):

$$\Delta G_{bind} = \Delta E_{bind} - T\Delta S \quad (2)$$

$$\Delta E_{bind} = \Delta E_{MM} + \Delta G_{sol} \quad (3)$$

*Reagents and cell lines*. Curcumin was obtained from Shanghai University of Traditional Chinese Medicine. All cell culture reagents were purchased from Biowest (USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) detection kit and Annexin V-FITC apoptosis detection kit were obtained from BestBio (Shanghai, China). Western blot antibodies specific to β-catenin and β-actin were ordered from Cell Signaling Technology (Beverly, MA, USA). BEL-7402 and QGY-7703 human HCC cell lines were provided by Shanghai Institute of Biochemistry and Cell Biology. They were cultured in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum, 1 x penicillin-streptomycin solution in a humidified 5% $CO_2$ atmosphere at 37˚C.

*Cell proliferation assay*. Cell proliferation was determined by MTT assay and used according to the manufacturer's instructions. Briefly, BEL-7402 and QGY-7703 cells were seeded into 96-well plates at a density of $5x10^3$ per well (100 $\mu$l). After 24 h, the indicated concentrations of curcumin were added. After incubating for 48 h, cells were washed twice with PBS. MTT medium (10 $\mu$l) were then added into each well, at which time cells were incubated for another 4 h. After which, the medium was removed, and 150 $\mu$l of dissolution was added into each well. The plate was gently rotated on an orbital shaker for 10 min to dissolve the precipitation completely. The absorbances were detected at 492 nm with a Microplate reader.

*Flow cytometry and apoptosis detection*. BEL-7402 and QGY-7703 cells were treated with curcumin in complete medium for 48 h as previously described. Following the treatment, cells were harvested by trypsin (not containing EDTA) and rinsed twice with PBS at 4˚C. Cells were then resuspended in 1X Annexin V binding buffer. A total of 5 $\mu$l of Annexin V-FITC solution was added to each tube. All tubes were incubated for 15 min at 4˚C in darkness. PI solution (15 $\mu$l) was added to each tube. All tubes were incubated for another 5 min at 4˚C in darkness. Cells were then analyzed using flow cytometry (Accuri C6, USA).

*Cell migration assay*. BEL-7402 and QGY-7703 cells were cultured with curcumin at various concentrations on 6-well plates, and after 24 h the cells were scratched in straight line with tips. The scratched cells were washed off with PBS and the resting cells were cultured in fresh medium: cell migrations were measured at 6, 12 and 24 h, respectively, using ImageJ software.

*Western blot analysis*. To detect protein expression and modification in response to treatment with curcumin, HCC cells were plated onto 6-well plates at a density of $2x10^5$ cells/ml and were then treated with various concentrations of curcumin. After incubating for 48 h, cells were lysed in cold RIPA lysis buffer. Total protein were extracted with high-salt buffer (0.5% sodium deoxycholate, 1% SDS, 1 mM sodium orthovanadate, 1 mM β-glycerol phosphate, 1 mM sodium fluoride, 2.5 mM sodium pyrophosphate) that contains a protease inhibitor cocktail (Roche, Nutley, NJ, USA). Protein samples were separated by SDS-PAGE, transferred onto PVDF membrane, and immunoblotted with the corresponding antibodies. The signal was visualized with Enhanced Chemiluminescence Plus (ECL Plus) detection system (Dingguo, China).

*RT-PCR*. The BEL-7402 cells and QGY-7703 cells were treated with curcumin at 10, 20, 40 and 80 $\mu$M for 48 h as previously described. Total RNAs were extracted from the cells using a commercially available RNA-Bee isolation kit (Tel-Test). Standard reverse transcriptions were performed with 500 ng of total RNA using TIANScript RT kit (Tiangen, Beijing, China). RT-PCRs were performed by using 1 $\mu$l of cDNA template, 10 pmol of primers, and a PCR premix (1 unit of Taq DNA polymerase, 250 mM dNTPs, 10 mM Tris-HCl, 40 mM KCl and

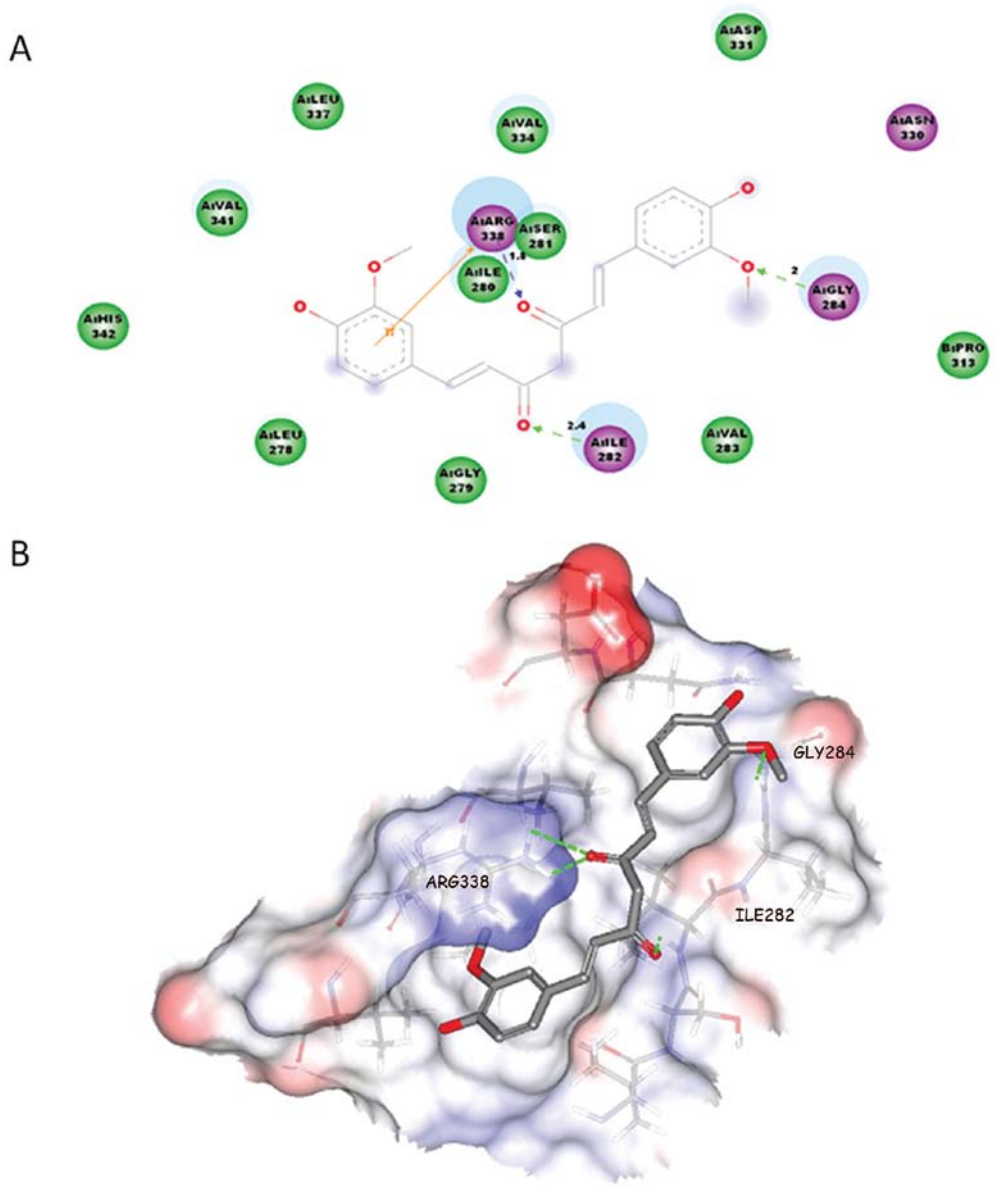


Figure 1. Molecular docking and binding free energy of curcumin with Dvl2-PDZ domain. (A) 2-D structure of curcumin interacting with Dvl2-PDZ domain. Curcumin is represented by line models and the atoms of carbon and oxygen of curcumin are represented by silver and red colorations, respectively; all critical residues in Dvl2-PDZ domains are represented in circles, of which major contributors for binding are shown in purple and minor contributors are shown in green. (B) The 3-D structure shows an electrostatic molecular surface model depicting curcumin binding to the Dvl2-PDZ domain: blue represents areas of positive-charge and red represents areas of negative-charge, and stick model depicts curcumin as well as critical residues of the Dvl2-PDZ domain; the atoms of carbon, oxygen and nitrogen of curcumin as well as critical amino acids are presented in silver, red and blue, respectively. Cation-π interactions between curcumin and ARG338 are shown in brown, and hydrogen bonds between curcumin and critcial resiudes including ARG338, ILE282 and GLY284 are presented as green dots.

1.5 mM $MgCl_2$; Tiangen). The following primers were used in the PCR reactions: c-myc forward, 5'-ctaccctctcaacgacagcag-3'; reverse, 5'gtgtgttcgcctcttgacatt-3'; VEGF forward, 5'-gcagaatca tcacgaagtggt-3'; reverse, 5'-catttgttgtgctgtaggaagc-3'; cyclin D1 forward, 5'-atctacaccgacaactccatcc-3'; reverse, 5'-gcattttggagag gaagtgttc-3'; β-actin forward, 5'-agagctacgagctgcctgctg-3'; reverse, 5'-agtacttgcgctcaggagga-3'.

The amplified products obtained from β-actin specific primers served as internal controls. PCRs were conducted using Bio-Rad T-100 (Bio-Rad, Hercules, CA, USA) with a denaturation step at 94˚C for 5 min; 30 cycles of 94˚C for 30 sec, 62˚C for 30 sec and 72˚C for 30 sec; and a final extension at 72˚C for 10 min. PCR amplifications were verified to be in the linear range.

*Statistical analysis*. Data were presented as mean ± SD for three separate experiments. One-way ANOVA was employed for statistical analysis using SPSS 17.0. $P<0.05$ was considered statistically significant.

## Results

*Molecular docking and binding free energy calculation between curcumin and Dvl2-PDZ domain*. The details of

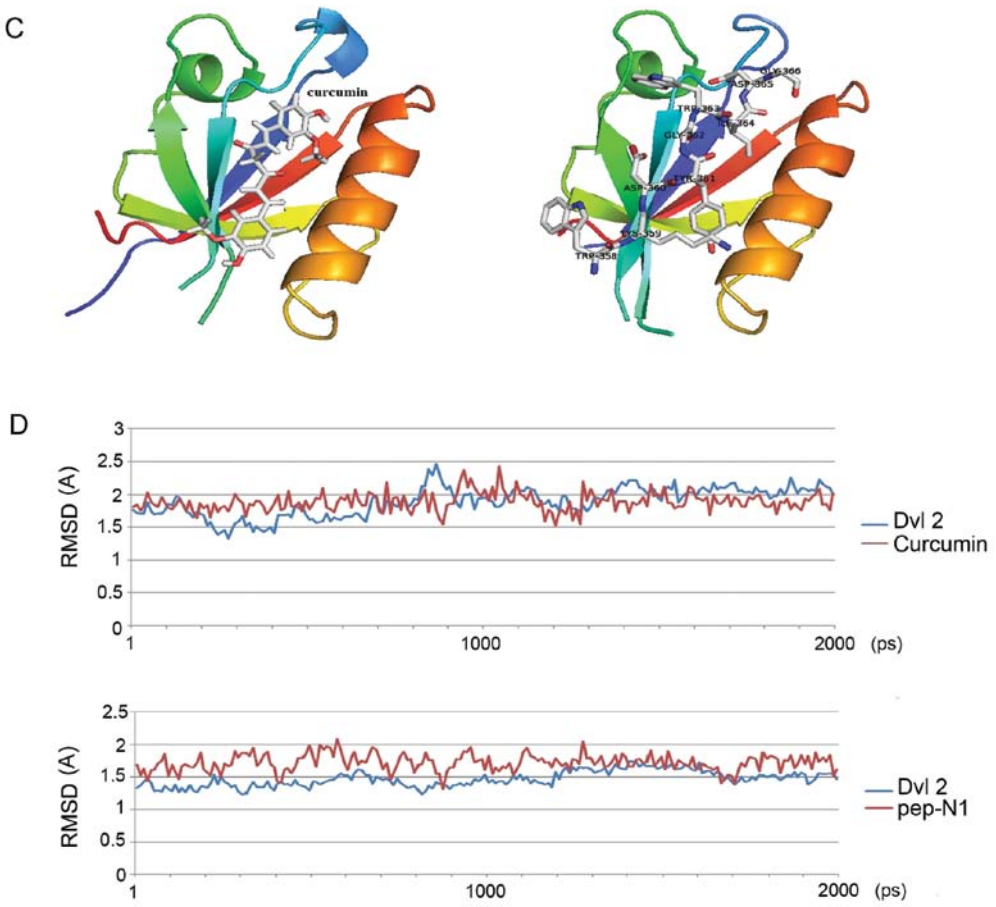


Figure 1. Continued. (C) The left side depicts the binding of curcumin to Dvl2-PDZ, and the right side the binding of inhibitory N1 peptide (pep-N1) to Dvl2-PDZ. (D) This figure shows the heavy atom RMSD of the last 2 nsec of each MD simulation.

Table I. Calculated binding free energy (kcal/mol) of protein/ligand complex.

| Complex | $\Delta E_{MM}$ | $\Delta G_{sol}$ | $-T\Delta S$ | $\Delta G_{bind}$ | $\Delta G_{bind/heavy\_atom}$[a] |
|---|---|---|---|---|---|
| Dvl2-PDZ/curcumin | -48.6382 | 30.2848 | 12.0148 | -6.3386 | -0.2348 |
| Dvl2-PDZ/pep-N1 | -293.878 | 228.554 | 41.9076 | -23.426 | -0.2856 |

[a]Calculated binding free energy divided by the number of heavy atoms of ligand.

interaction between curcumin and dishevelled2 (Dvl2) PDZ domain is shown in Fig. 1A. According to the figure, the two separated hydrogen atoms of -$NH_2$ group of ARG338 formed hydrogen bonds with the C=O group in curcumin and 2 (-$NH_2$) groups of ARG338 with positive charge had cation-π interaction with an aromatic ring of curcumin. The backbone -NH groups of ILE282 and GLY284 formed hydrogen bonds with the C=O and OH in curcumin, respectively. The configuration of curcumin fits into a hydrophobic pocket of Dvl2-PDZ domain that is mainly constructed by LEU278, LGY279, ILE280, SER281, VAL283, PRO313, ASP331, VAL334, LEU337, VAL341 and HIS342 in a shape-shape complimentary pattern, as shown in Fig. 1B. To evaluate the binding free energy of curcumin with Dvl2-PDZ domain, 3 nsec MD simulation was performed for each complex. We also calculated binding free energy of Dvl2-PDZ with a well-known inhibitory N1 peptide, pep-N1 (WKDYGWIDG) (26). Table I shows free energies of each items of protein in complex with curcumin and peptide based on the heavy atom RMSD of the last 2 nsec of each simulation. The RMSD values prove that the conformation of both complexes were stable.

*Curcumin suppresses BEL-7402 and QGY-7703 cell proliferation and induces apoptosis*. To determine potential cytotoxic and anti-proliferative effects of curcumin, the human HCC cell lines BEL-7402 and QGY-7703 were cultured with curcumin at various concentrations (0-80 $\mu$M). Cell viability was then determined by MTT assay. Results showed that treatment with curcumin led to a significant dose-dependent inhibition of HCC cell growth *in vitro*

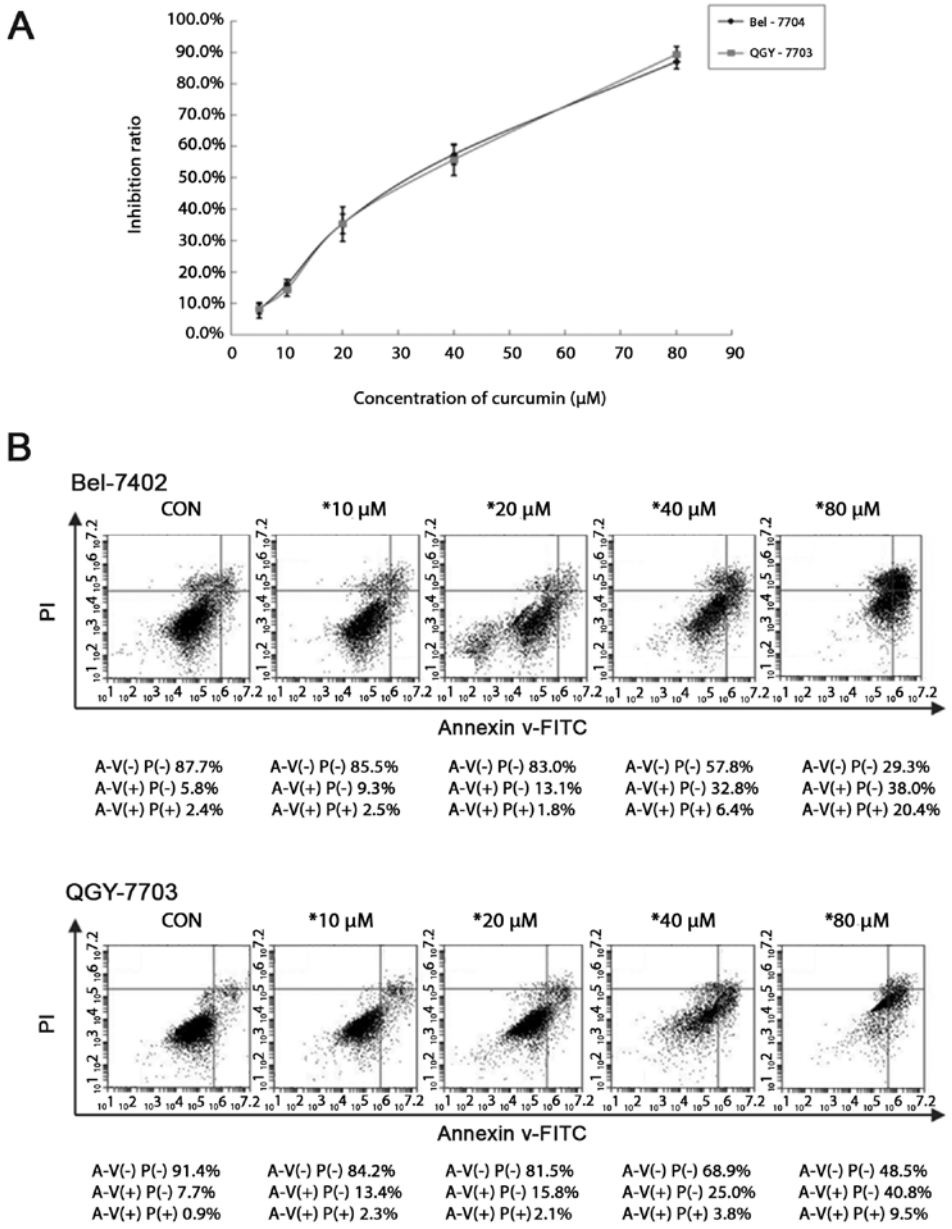


Figure 2. Curcumin suppressed HCC cells proliferation and induced cell apoptosis. The relative cell viability of Bel-7402 and QGY-7703 cells treated with different concentrations of curcumin was measured using MTT assay, and the inhibition ratios were calculated by normalization with the control group. The standard deviations were calculated based on three repeat experiments. (B) Bel-7402 and QGY-7703 cells were cultured with different concentrations of curcumin for 48 h, with the apoptotic fraction of cells detected by Annexin V staining (x-axis)/PI (y-axis) staining using flow cytometry. The lower right quadrant of the plot indicates early apoptotic cells that are Annexin V-positive and PI-negative.

(Fig. 2A). The half maximal (50%) inhibitory concentrations ($IC_{50}$) for BEL-7402 and QGY-7703 cells were approximately 32 and 33 $\mu$M, respectively.

Induction of cell apoptosis was confirmed by Annexin V-FITC staining in BEL-7402 and QGY-7703 cells. Results showed that treatment with curcumin led to significant dose-dependent apoptosis in HCC cells *in vitro*. According to Fig. 2B, the upper right quadrant represents late apoptosis, and the lower right quadrant represents early apoptosis. Increasing concentrations of curcumin, 0, 10, 20, 40 and 80 $\mu$M, respectively, were added to BEL-7402 and QGY-7703 cells for 48 h. As a result, the rates of cell apoptosis were 5.8, 9.3, 13.1, 32.8 and 38.0% for BEL-7402 and 7.7, 13.4, 15.8, 25.0 and 40.8% for QGY-7703, respectively. Thus, as the concentration of curcumin increased, the rates of apoptosis for BEL-7402 and QGY-7703 cells were also increased. The standard deviations were calculated based on three independent experiments.

*Curcumin suppressed BEL-7402 and QGY-7703 cell migration*. The inhibitory effect of curcumin for HCC cell migration was confirmed by cell migration test. After 24 h, cells migrated to the scratch area, which achieved healing in control group without the curcumin treatment. When treated with curcumin, especially at 80 $\mu$M, cells grew and migrated at a much slower pace (Fig. 3A). The width of the healewound

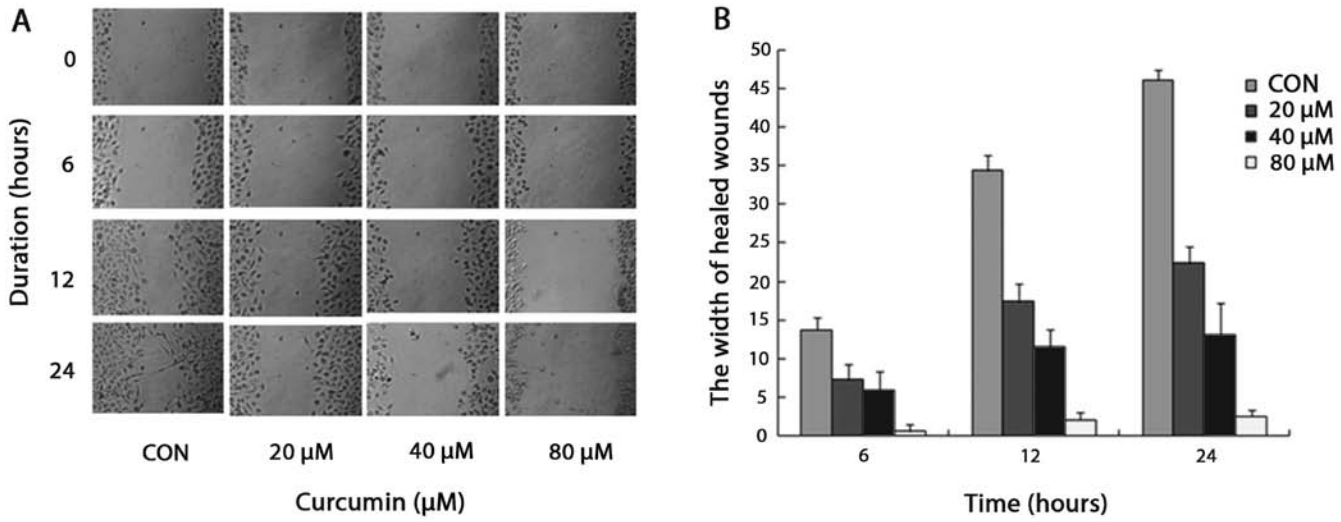


Figure 3. Curcumin inhibits HCC cell migration. (A) Bel-7402 and QGY-7703 cells were cultured with curcumin at various concentrations on 6-well plates, and after 24 h the cells were scratched in straight line with tips to make gaps of the same width. Phase contrast images of the same area of the gaps were taken at varying time intervals. (B) The width of healed wounds at varying time intervals was measured using ImageJ software. The standard deviations were calculated on three repeat experiments.

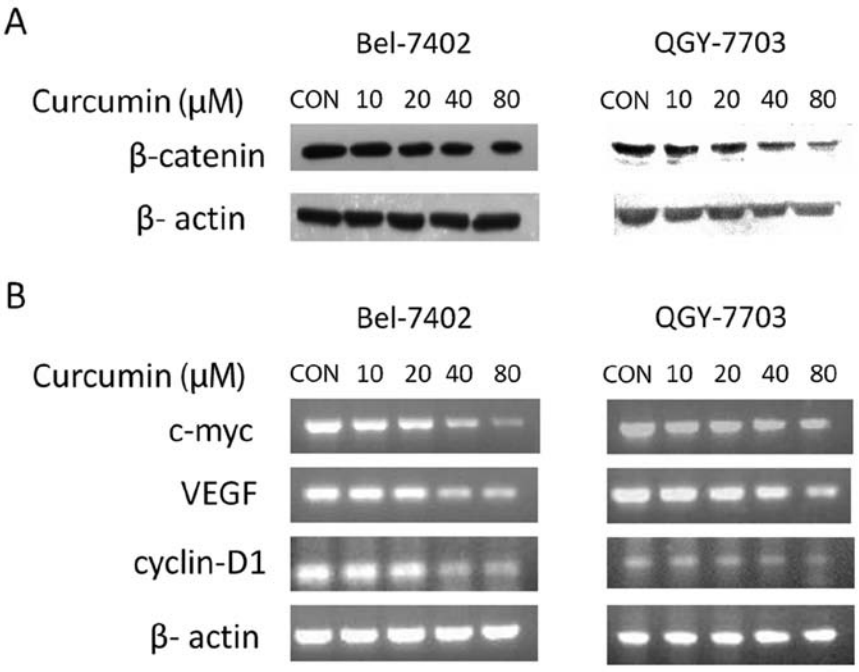


Figure 4. Curcumin inhibits β-catenin accumulation and suppresses the expression of target genes in HCC cells. Bel-7402 and QGY-7703 cells were treated with curcumin (0-80 $\mu$M) for 48 h. The expression of β-catenin was detected by western blot analysis. β-actin was used as a loading control. (B) The mRNA levels of c-myc, VEGF and cyclin D1 in Bel-7402 and QGY-7703 cells treated with curcumin were detected by RT-PCR. Housekeeping gene β-actin was used as a control.

was significantly different between groups treated with curcumin and the control group after 6 h, and the differences increased further after 12 and 24 h (Fig. 3B).

*Curcumin decreases β-catenin accumulation and down-regulates expression of target genes in BEL-7402 and QGY-7703 cells*. After being treated with curcumin, expression of β-catenin in BEL-7402 and QGY-7703 cells decreased in a dose-dependent pattern as shown by western blot analysis. As a result, β-catenin activity in these cells was reduced in general (Fig. 4A). Exposure to curcumin resulted in a dose-dependent decrease in the expression of cyclin D1 mRNA in both BEL-7402 and QGY-7703 cells, as demonstrated by RT-PCR experiments. Furthermore, the expressions of c-myc and VEGF was also significantly reduced at transcriptional level (Fig. 4B).

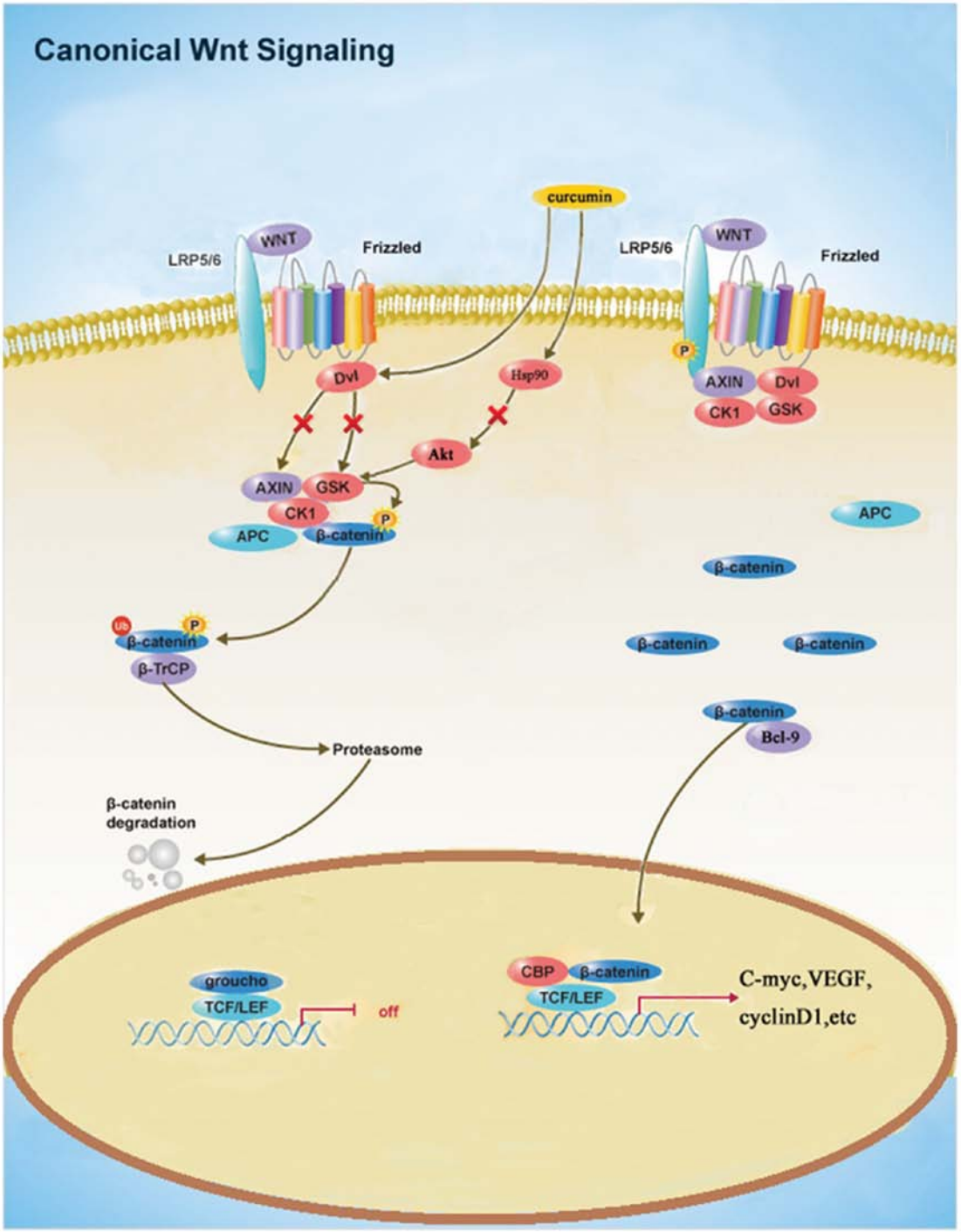


Figure 5. The mechanism of curcumin involved in the Wnt canonical signaling pathway. Wnt ligands bind to Frizzled receptor and LRP5/6 co-receptors, trigger the cell membrane recruitment of Dvl which in turn recruit axin, CK1α and GSK3β to the membrane, induce the accumulation of free β-catenin in the cytoplasm. The nuclear translocation of β-catenin will turn on the expression of target genes. When curcumin binds to Dvl, it will inhibit the recruitment of axin, CK1α and GSK3β to the membrane. Cucumin might also bind to Hsp90, dephosphorylate Akt and activate GSK3β to maintain the β-catenin destruction complex. Both bindings by curcumin will set the cell in Wnt 'off-state' suppressing the nucleus accumution of β-catenin and the expression of Wnt target genes.

## Discussion

In the absence of wnt ligands, newly synthesized β-catenin forms a complex in the cytosol with axin, adenomatous polyposis coli (APC), glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α). In this complex, β-catenin is constitutively phosphorylated by CK1α and subsequently by GSK3β. The phosphorylated β-catenin is recognized by the ubiquitin ligase β-transducin-repeat containing protein (β-Trcp), targeted to the proteasome pathway and degraded by 26S proteasome (1). Thus, free β-catenin is maintained at a low level in the cytoplasm, suppressing the expression of many target genes that are important for cell survival and proliferation. When the wnt ligand binds to wnt receptor, the ligand-receptor complex will recruit dishevell (Dvl) and axin from cytosol to the membrane. The binding of Dvl with axin

then recruits CK1α and GSK3β to LRP5/6, which consequently inhibits the formation of the β-catenin destruction complex (27,28). As a result, the accumulated free β-catenin in cytoplasm is recruited to the nucleus by Bcl-9/legless (Bcl-9) adaptor protein and binds to the Tcf/Lef transcription factor, which in turn activates the expression of wnt target genes involved in cell proliferation and survival (29).

To study the mechanism of curcumin inhibitory effects on carcinoma cells, molecular docking simulation was performed. The docking results suggest that curcumin has strong interactions with Dvl2-PDZ domain, which involves cation-π interaction, salt-bridge bond and multiple backbone interactions. We then calculated binding free energy between curcumin and Dvl2-PDZ domain. Based on negative total binding free energy -6.3386 kcal/mol, it was clearly seen that Dvl2-PDZ/curcumin is a favorable protein/ligand complex. Though the binding energy of curcumin is weaker than that of pep-N1 with the nine residues, the $\Delta G_{bind/heavy\ atom}$ of curcumin is nearly equal to that of pep-N1. These results suggest that the interaction between curcumin and Dvl2-PDZ domain results in curcumin suppressing Dvl2 activities and subsequently preventing axin recruitment to cellular membrane, which eventually results in the phosphorylation of β-catenin through the destruction complex and digestion by the proteasome system.

We confirmed that curcumin significantly inhibited carcinoma cell growth *in vitro* in two human HCC cell lines, BEL-7402 and QGY-7703, shown in dose-dependent pattern using MTT assay. Additionally, curcumin interrupted Wnt signaling pathway and decreased β-catenin activity in a dose-dependent manner, as shown by western blot analysis. HCC cells treated with curcumin resulted in significant transcriptional decrease of many genes related to cell growth, survival and apoptosis, including c-myc, VEGF and cyclin D1, by RT-PCR experiment. This in turn led to HCC cell apoptosis and prevented HCC cell migration, as demonstrated by apoptosis assay and cell migration assay, respectively.

Our data suggest that anti-proliferative function of curcumin results from curcumin inhibition of wnt/β-catenin signaling pathway as shown in the diagram (Fig. 5). Based on our conclusion, the binding of curcumin to Dvl2 helps the formation of the β-catenin destruction complex, which turns the Wnt signaling pathway to 'off state' by decreasing free β-catenin levels in the cytoplasm. However, it was reported that curcumin can bind to Hsp90 proteins (30). One group indicated that the Hsp90 inhibitor can dephosphorylate Akt and activate GSK3β (12). Therefore, curcumin might regulate the β-catenin destruction complex in two different ways and attenuate the wnt signals in carcinoma cells, but there is still a long way to go before the mechanism of curcumin is fully understood so that it would be beneficial for the patients with HCC.

## Acknowledgements

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## References

1. Aberle H, Bauer A, Stappert J, Kispert A and Kemler R: beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J 16: 3797-3804, 1997.
2. Baeg GH and Perrimon N: Functional binding of secreted molecules to heparan sulfate proteoglycans in *Drosophila*. Curr Opin Cell Biol 12: 575-580, 2000.
3. Banerjee M, Tripathi LM, Srivastava VM, Puri A and Shukla R: Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. Immunopharmacol Immunotoxicol 25: 213-224, 2003.
4. Case DA, Darden TA, Cheatham TE III, *et al*: Wnt/beta-catenin signaling in development and disease. Cell 127: 469-480, 2012.
5. Daugherty RL and Gottardi CJ: Phospho-regulation of Beta-catenin adhesion and signaling functions. Physiology 22: 303-309, 2007.
6. Giommarelli C, Zuco V, Favini E, Pisano C, Dal Piaz F, De Tommasi N and Zunino F: The enhancement of antiproliferative and proapoptotic activity of HDAC inhibitors by curcumin is mediated by Hsp90 inhibition. Cell Mol Life Sci 67: 995-1004, 2010.
7. Guillen-Ahlers H: Wnt signaling in renal cancer. Curr Drug Targets 9: 591-600, 2008.
8. Itasaki N and Hoppler S: Crosstalk between Wnt and bone morphogenic protein signaling: a turbulent relationship. Dev Dyn 239: 16-33, 2010.
9. Janssens N, Janicot M and Perera T: The Wnt-dependent signaling pathways as target in oncology drug discovery. Invest New Drugs 24: 263-280, 2006.
10. Kakarala M, Brenner DE, Korkaya H, *et al*: Targeting breast stem cells with the cancer preventive compounds curcumin and piperine. Breast Cancer Res Treat 122: 777-785, 2010.
11. Kohn AD and Moon RT: Wnt and calcium signaling: beta-catenin-independent pathways. Cell Calcium 38: 439-446, 2005.
12. Kurashina R, Ohyashiki JH, Kobayashi C, Hamamura R, Zhang Y, Hirano T and Ohyashiki K: Anti-proliferative activity of heat shock protein (Hsp) 90 inhibitors via beta-catenin/TCF7L2 pathway in adult T cell leukemia cells. Cancer Lett 284: 62-70, 2009.
13. Lammi L, Arte S, Somer M, *et al*: Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer. Am J Hum Genet 74: 1043-1050, 2004.
14. Laurent-Puig P, Legoix P, Bluteau O, *et al*: Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology 120: 1763-1773, 2001.
15. Li Y, Kong D, Wang Z, Ahmad A, Bao B, Padhye S and Sarkar FH: Inactivation of AR/TMPRSS2-ERG/Wnt signaling networks attenuates the aggressive behavior of prostate cancer cells. Cancer Prev Res (Phila) 4: 1495-1506, 2011.
16. Logan CY and Nusse R: The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 20: 781-810, 2004.
17. Miquel J, Bernd A, Sempere JM, Diaz-Alperi J and Ramirez A: The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. Arch Gerontol Geriatr 34: 37-46, 2002.
18. Mosimann C, Hausmann G and Basler K: Beta-catenin hits chromatin: regulation of Wnt target gene activation. Nat Rev Mol Cell Biol 10: 276-286, 2009.
19. Niehrs C and Acebron SP: Mitotic and mitogenic Wnt signalling. EMBO J 31: 2705-2713, 2012.
20. Nusse R: Wnt signaling in disease and in development. Cell Res 15: 28-32, 2005.
21. Peifer M and Polakis P: Wnt signaling in oncogenesis and embryogenesis - a look outside the nucleus. Science 287: 1606-1609, 2000.
22. Prasad CP, Rath G, Mathur S, Bhatnagar D and Ralhan R: Potent growth suppressive activity of curcumin in human breast cancer cells: modulation of Wnt/beta-catenin signaling. Chem Biol Interact 181: 263-271, 2009.
23. Shiina H, Igawa M, Breault J: The human T-cell factor-4 gene splicing isoforms, Wnt signal pathway, and apoptosis in renal cell carcinoma. Clin Cancer Res 9: 2121-2132, 2003.
24. Zhang Y, Appleton BA, Wiesmann C, Lau T, Costa M, Hannoush RN and Sidhu SS: Inhibition of Wnt signaling by disheveled PDZ peptides. Nat Chem Biol 5: 217-219, 2009.

25. Sun L, Tian Z and Wang J: A direct cross-talk between interferon-gamma and sonic hedgehog signaling that leads to the proliferation of neuronal precursor cells. Brain Behav Immun 24: 220-228, 2010.
26. Veeman MT, Axelrod JD and Moon RT: A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. Dev Cell 5: 367-377, 2003.
27. Vivekanand P and Rebay I: Intersection of signal transduction pathways and development. Annu Rev Genet 40: 139-157, 2006.
28. Yamamoto H, Oue N, Sato A, Hasegawa Y, Matsubara A, Yasui W and Kikuchi A: Wnt5a signaling is involved in the aggressiveness of prostate cancer and expression of metalloproteinase. Oncogene 29: 2036-2046, 2010.
29. Zeng X, Huang H, Tamai K, *et al*: Initiation of Wnt signaling: control of Wnt coreceptor Lrp6 phosphorylation/activation via frizzled, disheveled and axin functions. Development 135: 367-375, 2008.
30. Zeng X, Tamai K, Doble B, *et al*: A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. Nature 438: 873-877, 2005.