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Original Research Article

Anti-Proliferative Activity of Ethanol Extracts from Taxilli Ramulus (*Taxillus chinensis* (DC.) Danser) Through Cyclin D1 Proteasomal Degradation in Human Colorectal Cancer Cells

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Abstract - In this study, we elucidated anti-cancer activity and potential molecular mechanism of 70% ethanol extracts from Taxilli Ramulus (*Taxillus chinensis* (DC.) Danser) (TR-E70) against human colorectal cancer cells. Anti-cell proliferative effect of TR-E70 was evaluated by MTT assay. The effect of TR-E70 on the expression of cyclin D1 in the protein and mRNA level was evaluated by Western blot and RT-PCR, respectively. TR-E70 suppressed the proliferation of human colorectal cancer cell lines, HCT116 and SW480. Although TR-E70 decreased cyclin D1 expression in protein and mRNA level, decreased level of cyclin D1 protein by TR-E70 more dramatically occurred than that of cyclin D1 mRNA. Cyclin D1 downregulation by TR-E70 was attenuated in presence of MG132. In addition, TR-E70 phosphorylated threonine-286 (T286) of cyclin D1. TR-E70-mediated cyclin D1 degradation was blocked in presence of LiCl as an inhibitor GSK3β but not PD98059 as an ERK1/2 inhibitor and SB203580 as a p38 inhibitor. Our results suggest that TR-E70 may downregulate cyclin D1 as one of the potential anti-cancer targets through GSK3β-dependent cyclin D1 degradation. From these findings, TR-E70 has potential to be a candidate for the development of chemoprevention or therapeutic agents for human colorectal cancer.

Key words - Anticancer activity, Cancer chemoprevention, Cyclin D1, Human colorectal cancer, Taxilli Ramulus, *Taxillus chinensis* (DC.) Danser

Introduction

Herbal medicines have been attracted as the numerous candidates for the drug discovery because of their diverse bioactivities (Baker *et al*., 2007; Cragg *et al*., 1997). Taxilli Ramulus (TR, *Taxillus chinensis* (DC.) Danser) called as "Sangjisheng" has been used for treating rheumatoid arthralgia, threatened abortion and hypertension (Wang *et al*., 2008). In the pharmacological studies, TR has been reported to inhibit SARS-CoV replication and to inhibit fatty acid synthase resulting to body weight (Wang *et al*., 2008; Wen *et al*., 2011). It has been reported that TR has immunomodulatory activity, antioxidant activity, anti-inflammatory activity and anti-obesity activity (Zhang *et al*., 2013; Zhang *et al*., 2011). In addition, TR has been reported to contain a variety of anticancer compounds such as rutin, quercetin and phlorin, which has been used for the cancer treatment (Zhang *et al*., 2013). In our screening study for anticancer activity of the herbal medicines against human colorectal cancer cells, we observed that TR shows the remarkable anti-proliferative activity. Thus, we chased TR for the further study.

Human colorectal cancer has been regarded as one of the most common cancers and a major cause of death worldwide (Siegel *et al*., 2017). Twenty percentage of human colorectal cancer has already reached distant metastasis at the time of diagnosis and the 5-year survival rate of metastatic human colorectal cancer ranged from 5.5% to 12.5% (Woo and Jung, 2017). Among the molecular pathology associated with human colorectal cancer, the upregulation of cyclin D1 is

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involved in pathogenesis and metastases of human colorectal cancer (Alao, 2007; Mermelshtein *et al*., 2005) and observed in 68.3% of human colorectal cancer cases (Bahnassy *et al*., 2004; Holland *et al*., 2001). Thus, cyclin D1 has been regarded as an potential chemopreventive and therapeutic target for the development of anti-cancer agents (Musgrove *et al*., 2011).

In this study, we evaluated anti-proliferative effect of TR and elucidated that TR downregulates cyclin D1 protein level in human colorectal cancer cells. We herein reports that TR attenuates cyclin D1 protein level through the induction of cyclin D1 proteasomal degradation, which results in the suppression of the cell proliferation in human colorectal cancer cells.

Materials and Methods

Materials

Cell culture media, Dulbecco's Modified Eagle medium (DMEM)/F-12 1:1 Modified medium (DMEM/F-12) was purchased from Lonza (Walkersville, MD, USA). PD98059, SB203580, LiCl, MG132 and 3-(4,5-dimethylthizaol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Aldrich (St. Louis, MO, USA). Antibodies against cyclin D1 (#9336), phospho-cyclin D1 (Thr286) (#3300) and β-actin (#5152) were purchased from Cell Signaling (Bervely, MA, USA). All chemicals were purchased from Fisher Scientific, unless otherwise specified.

Sample preparation

Taxilli Ramulus (TR, *Taxillus chinensis* (DC.) Danser) was purchased from Humanherb, Korea and formally identified by Jin Suk Koo as the professor of Andong National University, Korea. Two hundred gram of TR was extracted with l L of 70% ethanol with shaking for 48 h. After 48 h, the ethanol-soluble fraction was filtered and concentrated to approximately 300 ㎖ volume using a vacuum evaporator and then freeze-dried. The ethanol extracts (TR-E70) from TR was kept in a refrigerator until use.

Cell culture and treatment

Human colorectal cancer cell lines such as HCT116 and SW480 were purchased from Korean Cell Line Bank (Seoul, Korea) and grown in DMEM/F-12 supplemented with 10% fatal bovine serum (FBS), 100 U/㎖ penicillin and 100 ㎍/㎖ streptomycin. The cells were maintained at 37℃ under a humidified atmosphere of 5% CO_2. TR-E70 was dissolved in dimethyl sulfoxide (DMSO) and treated to cells. DMSO was used as a vehicle and the final DMSO concentration did not exceed 0.1% (v/v).

Cell proliferation assay

Anti-proliferative effect of TR-E70 was investigated by MTT assay according to the literatures (Park *et al*., 2016b; Park *et al*., 2016c). Briefly, HCT116 and SW480 cells were seeded onto 96-well plate for 24 h. The cells were dose-dependently treated with TR-E70 for 24 h. Then, the cells were incubated with 50 ㎕ of MTT solution (1 ㎎/㎖) for an additional 2 h. After 2 h, the resulting crystals were dissolved in DMSO. The formation of formazan was measured by reading absorbance at a wavelength of 570 ㎚.

SDS-PAGE and Western blot

SDS-PAGE and Western blot was performed according to the literatures (Park *et al*., 2016b; Park *et al*., 2016c). After TR-E70 treatment, cells were washed with 1 × phosphate-buffered saline (PBS) two times, and lysed in radioimmuno-precipitation assay (RIPA) buffer (Boston Bio Products, Ashland, MA, USA) containing protease inhibitor cocktail (Sigma-Aldrich) and phosphatase inhibitor cocktail (Sigma-Aldrich) for 30 min in 4℃. After 30 min, the cell lysates were centrifuged at 15,000 × rpm for 10 min at 4℃. Protein concentration from the cell lysates was measured by the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA). The equal amount of proteins from the cell lysates were subjected to SDS-PAGE and transferred to PVDF membrane (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were blocked for non-specific binding with 5% non-fat dry milk in Tris-buffered saline containing 0.05% Tween 20 (TBS-T) for 1 h at room temperature, and then incubated with specific primary antibodies in 5% non-fat dry milk at 4℃ overnight. After three washes with TBS-T, the membranes were incubated with horse radish peroxidase (HRP)-conjugated immunoglobulin G (IgG) for 1 h at room temperature and chemiluminescence was detected with ECL

Western blotting substrate (Amersham Biosciences, Piscataway, NJ, USA) and visualized in Polaroid film.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

RT-PCR was performed according to the literatures (Park *et al*., 2016b; Park *et al*., 2016c). After TR-E70 treatment, total RNA from the cells was extracted using a RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and then 1 ㎍ of total RNA was reverse-transcribed using a Verso cDNA Kit (Thermo Scientific, Pittsburgh, PA, USA) according to the manufacturer's protocol for cDNA synthesis. PCR was performed using PCR Master Mix Kit (Promega, Madison, WI, USA) with human primers for cyclin D1 and GAPDH as followed : cyclin D1: forward 5'-aactacctggaccgcttcct-3' and reverse 5'-ccacttgagcttgttcacca-3', GAPDH: forward 5'-acccagaaga ctgtggatgg-3' and reverse 5'-ttctagacggcaggtcaggt-3'. The following PCR reaction conditions were used: 1 cycle of (3 min at 94℃ for denaturation), 25 cycles of (30 s at 94℃ for denaturation, 30 s at 60℃ for annealing, and 30 s at 72℃ for elongation), and 1 cycle of (5 min for extension at 72℃).

Statistical analysis

All the data are shown as mean ± SEM (standard error of mean). Statistical analysis was performed with one-way ANOVA followed by Dunnett's test. Differences with $*P < 0.05$ were considered statistically significant.

Results and Discussion

Effect of TR-E70 on the cell proliferation in human colorectal cancer cell

The abnormal cell proliferation has been reported to be associated with the cancer development. Thus, we evaluated whether TR-E70 exerts anti-proliferative effect against human colorectal cancer cells. As shown in Fig. 1A and 1B, TR-E70 suppressed the cell proliferation by 35.1% and 16.7% at 25 ㎍/㎖, 57.1% and 47.1% at 50 ㎍/㎖, and 75.1% and 71.5% at 100 ㎍/㎖ in HCT116 (APC-wild type) and SW480 cells (APC-mutant), respectively. These data indicate that TR-E70 suppressed cell growth of human colorectal cancer cells in dose-dependent manner and anti-proliferative activity of TR-E70 is APC-independent.

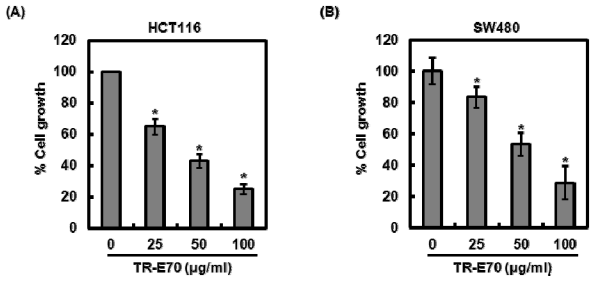

Fig. 1. Inhibitory effect of TR-E70 on the proliferation of human colorectal cancer cell lines, HCT116 and SW480. (A, B) The cells were plated overnight and then treated with TR-E70 for 24 h. Cell proliferation was measured using MTT assay as described in Materials and methods. $*P < 0.05$ compared to cell without TR-E70 treatment.

Effect of TR-E70 on cyclin D1 level in human colorectal cancer cells

Among the factors regulating the cell proliferation, cyclin D1 as one of the oncogenic protein facilitates the cell cycle progression by complexing with cyclin-dependent kinase 4 and 6 (CDK4 and CDK6) (Alao, 2007). Thus, cyclin D1 has been regarded as an attractive molecular target in the development of anti-cancer drugs (Musgrove *et al*., 2011). Thus, a number of the natural products downregulating cyclin D1 protein level has been searched (Eo *et al*., 2015; Kato *et al*., 2015; Kim *et al*., 2015; Lee *et al*., 2008; Park *et al*., 2016a; Park *et al*., 2016b). In this study, we investigated whether TR-E70 affects cyclin D1 level in human colorectal cancer cell lines, HCT116 and SW480. As shown in Fig. 2A and 2B, TR-E70 dose-dependently decreased cyclin D1 protein level in HCT116 and SW480 cells. In RT-PCR analysis of cyclin D1 to determine whether TR-E70-mediated decreased level of cyclin D1 results from the transcriptional regulation (Fig. 2C and 2D), we observed that TR-E70 slightly reduced the level of cyclin D1 mRNA at 100 ㎍/㎖ in HCT116 and SW480 cells, which indicates that the transcriptional inhibition of cyclin D1 by TR-E70 may partially contribute to TR-E70- mediated downregulation of cyclin D1 protein. In time course experiment (Fig. 2E), cyclin D1 protein level started to be decreased at 10 h or 3 h after TR-E70 treatment in HCT116 and SW480 cells, respectively.

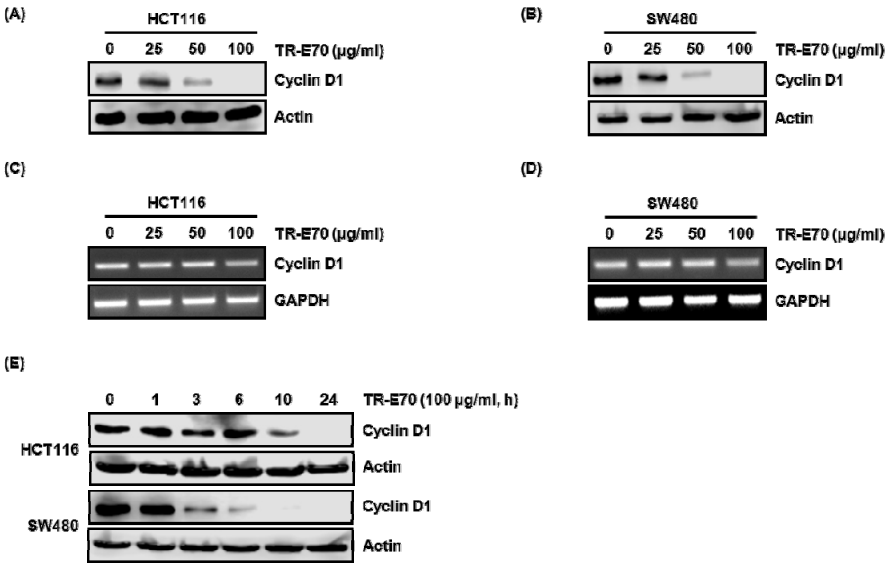

Fig. 2. Effect of TR-E70 on the cyclin D1 level in human colorectal cancer cells. (A, B) The cells were plated overnight and then treated with TR-E70 for 24 h. The cell lysates were subjected to SDS-PAGE and the Western blot was performed using antibody against cyclin D1. Actin was used as internal control for Western blot analysis. (C, D) The cells were plated overnight and then treated with TR-E70 for 24 h. For the analysis of cyclin D1 mRNA using RT-PCR, total RNA was prepared after TR-E70 treatment. GAPDH was used as internal control for RT-PCR. (E) HCT116 cells were treated with 100 ㎍/㎖ of TR-E70 for the indicated times. The cell lysates were subjected to SDS-PAGE and the Western blot was performed using antibody against cyclin D1. Actin was used as internal control for Western blot analysis.

Induction of cyclin D1 proteasomal degradation by TR-E70 in human colorectal cancer cells

In Fig. 2A-2D, we observed that 50 ㎍/㎖ of TR-E70 dramatically decreased the protein level of cyclin D1, while the mRNA level of cyclin D1 was not changed in the same concentration of TR-E70, which indicates that TR-E70 may reduce the protein stability of cyclin D1.

The upregulation of cyclin D1 protein does not occur solely as a consequence of cyclin D1 gene amplification. Actually, there is growing evidence that cyclin D1 gene amplification has been reported to account for 2.5% in human colorectal cancer, while the upregulation of cyclin D1 protein level has been observed by 55% in human colorectal cancer (Musgrove *et al*., 2011), which indicates that the upregulation of cyclin D1 protein level frequently is attributed to its defective regulation at the post-translational level (Gillett *et al*., 1994; Russell *et al*., 1999). Thus, we investigated whether TR-E70 mediates cyclin D1 proteasomal degradation in HCT116 and SW480 cells. As shown in Fig. 3A and 3B, we observed that MG132 attenuates the downregulation of cyclin D1 by TR-E70 in HCT116 and SW480 cells, respectively. In addition, we determined that TR-E70 mediates the threonine-286 (T286) phosphorylation of cyclin D1 because cyclin D1 degradation can be regulated by cyclin D1 T286 phosphorylation (Alao, 2007). As shown in Fig. 3C, TR-E70 induced T286 phosphorylation of cyclin D1 in HCT116 and SW480 cells, respectively. These data indicate that TR-E70 may induce cyclin D1 proteasomal degradation through phosphorylating cyclin D1 T286.

Dependency on GSK3β in TR-E70-mediated proteasomal degradation of cyclin D1 in human colorectal cancer cells

Cyclin D1 proteasomal degradation has been reported to be regulated by a variety of the upstream kinases such as ERK1/2, p38 and GSK3β (Alt *et al*., 2000; Casanovas *et al*., 2000; Diehl *et al*., 1998; Okabe *et al*., 2006; Thoms *et al*., 2007). Thus, the cells were pretreated with PD98059 (ERK1/2 inhibitor), SB203580 (p38 inhibitor) or LiCl (GSK3β inhibitor) and then co-treated with TR-E70. As shown in Fig. 4A and 4B, TR-E70 reduced cyclin D1 protein level in

absence or presence of PD98059 and SB203580, which indicates that TR-E70-mediated reduction of cyclin D1 protein level may be independent on ERK1/2 and p38. However, LiCl treatment reduced decrease of cyclin D1 protein induced by TR-E70 (Fig. 4C). These data suggest that TR-E70-mediated cyclin D1 proteasomal degradation may be dependent on GSK3β.

In conclusion, TR-E70 may downregulate the cyclin D1 level through inducing GSK3β-dependent proteasomal degradation, which may contribute to the inhibition of the

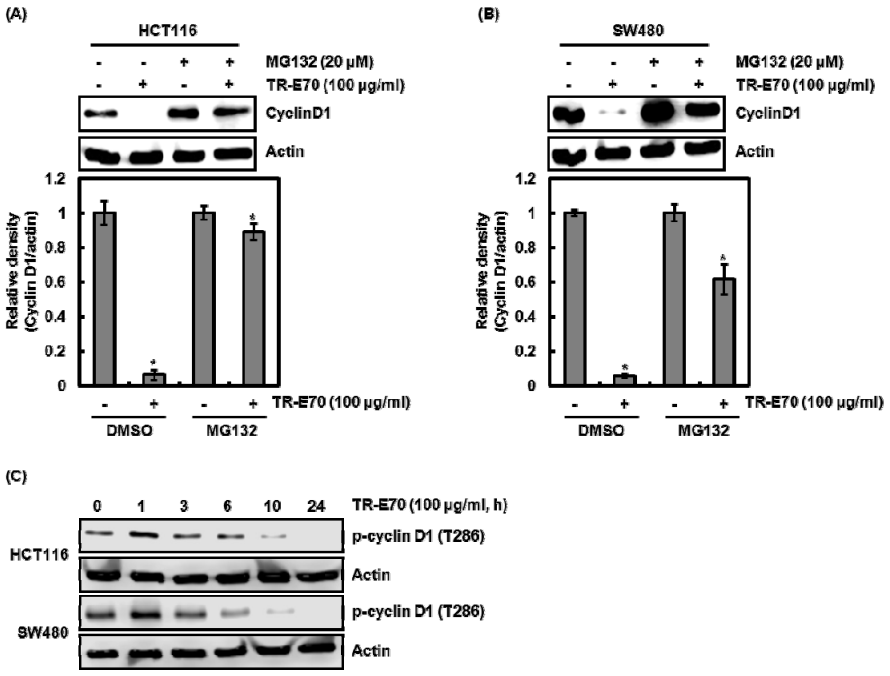

Fig. 3. Effect of TR-E70 on cyclin D1 proteasomal degradation. (A, B) HCT116 and SW480 cells were pretreated with MG132 for 2 h, and then co-treated with TR-E70 (100 ㎍/㎖) for 10 h and 3 h, respectively. Cell lysates were subjected to SDS-PAGE and the Western blot was performed using antibody against cyclin D1. (C) HCT116 and SW480 cells were treated with TR-E70 (100 ㎍/㎖) for the indicated times. Cell lysates were subjected to SDS-PAGE and the Western blot was performed using antibody against phospho-cyclin D1 (T286). Actin was used as internal control for Western blot analysis.

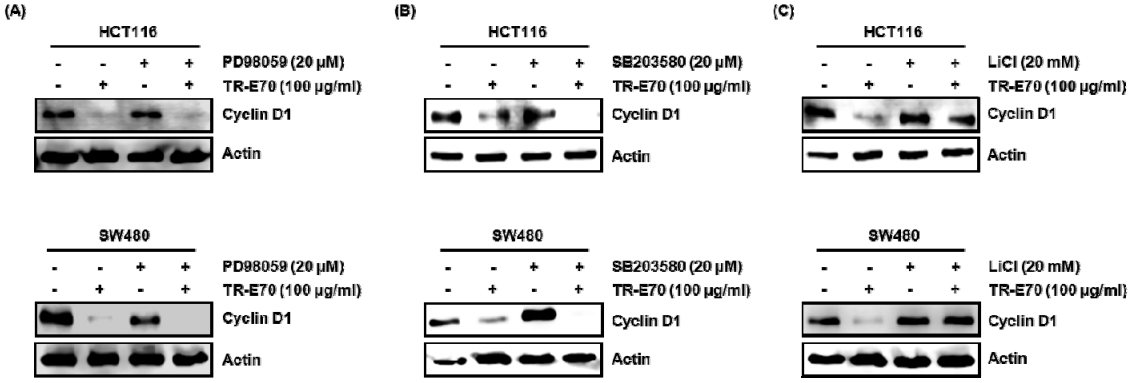

Fig. 4. Determination of the upstream kinases involved in TR-E70-mediated cyclin D1 proteasomal degradation. (A-C) HCT116 and SW480 cells were pretreated with PD98059 as an ERK1/2 inhibitor, SB203580 as a p38 inhibitor or LiCl as a GSK3β inhibitor for 2 h, and then co-treated with TR-E70 (100 ㎍/㎖) for 10 h and 3 h, respectively. Cell lysates were subjected to SDS-PAGE and the Western blot was performed using antibody against cyclin D1. Actin was used as internal control for Western blot analysis.

proliferation in human colorectal cancer cells. These findings can provide detailed account of preclinical studies conducted to determine the utility of TR-E70 as a therapeutic and chemopreventive agent for the treatment of human colorectal cancer.

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References

Alao, J.P. 2007. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. Mol. Cancer 6:24.

Alt, J.R., J.L. Cleveland, M. Hannink and J.A. Diehl. 2000. Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. Genes Dev. 14:3102-3114.

Bahnassy, A.A., A.R. Zekri, S. El-Houssini, A.M. El-Shehaby, M.R. Mahmoud, S. Abdallah and M. El-Serafi. 2004. Cyclin A and cyclin D1 as significant prognostic markers in colorectal cancer patients. BMC Gastroenterol. 23:22-24.

Baker, D.D., M. Chu, U. Oza and V. Rajgarhia. 2007. The value of natural products to future pharmaceutical discovery. Nat. Prod. Rep. 24:1225-1244.

Casanovas, O., F. Miro, J.M. Estanyol, E. Itarte, N. Agell and O. Bachs. 2000. Osmotic stress regulates the stability of cyclin D1 in a p38SAPK2-dependent manner. J. Biol. Chem. 275:35091-35097.

Cragg, G.M., D.J. Newman and K.M. Snader. 1997. Natural products in drug discovery and development. J. Nat. Prod. 60:52-60.

Diehl, J.A., M. Cheng, M.F. Roussel and C.J. Sherr. 1998. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. Genes Dev. 12:3499-3511.

Eo, H.J., G.H. Park, H.M. Song, J.W. Lee, M.K. Kim, M.H. Lee, J.R. Lee, J.S. Koo and J.B. Jeong. 2015. Silymarin induces cyclin D1 proteasomal degradation via its phosphorylation of threonine-286 in human colorectal cancer cells. Int. Immunopharmacol. 24:1-6.

Gillett, C., V. Fantl, R. Smith, C. Fisher, J. Bartek, C. Dickson, D. Barnes and G. Peters. 1994. Amplification and over-expression of cyclin D1 in breast cancer detected by immunohistochemical staining. Cancer Res. 54:1812-1817.

Holland, T.A., J. Elder, J.M. McCloud, C. Hall, M. Deakin, A.A. Fryer, J.B. Elder and P.R. Hoban. 2001. Subcellular localisation of cyclin D1 protein in colorectal tumours is associated with p21(WAF1/CIP1) expression and correlates with patient survival. Int. J. Cancer 95:302-306.

Kato, A., A. Naiki-Ito, T. Nakazawa, K. Hayashi, I. Naitoh, K. Miyabe, S. Shimizu, H. Kondo, Y. Nishi, M. Yoshida, S. Umemura, Y. Hori, T. Mori, M. Tsutsumi, T. Kuno, S. Suzuki, H. Kato, H. Ohara, T. Joh and S. Takahashi. 2015. Chemopreventive effect of resveratrol and apocynin on pancreatic carcinogenesis via modulation of nuclear phosphorylated GSK3beta and ERK1/2. Oncotarget 6:42963-42975.

Kim, M.K., G.H. Park, H.J. Eo, H.M. Song, J.W. Lee, M.J. Kwon, J.S. Koo and J.B. Jeong. 2015. Tanshinone I induces cyclin D1 proteasomal degradation in an ERK1/2 dependent way in human colorectal cancer cells. Fitoterapia 101: 162-168.

Lee, S.H., M. Cekanova and S.J. Baek. 2008. Multiple mechanisms are involved in 6-gingerol-induced cell growth arrest and apoptosis in human colorectal cancer cells. Mol. Carcinogenesis 47:197-208.

Mermelshtein, A., A. Gerson, S. Walfisch, B. Delgado, G. Shechter-Maor, J. Delgado, A. Fich and L. Gheber. 2005. Expression of D-type cyclins in colon cancer and in cell lines from colon carcinomas. Brit. J. Cancer 93:338-345.

Musgrove, E.A., C.E. Caldon, J. Barraclough, A. Stone and R.L. Sutherland. 2011. Cyclin D as a therapeutic target in cancer. Nat. Rev. Cancer 11:558-572.

Okabe, H., S.H. Lee, J. Phuchareon, D.G. Albertson, F. McCormick and O. Tetsu. 2006. A critical role for FBXW8 and MAPK in cyclin D1 degradation and cancer cell proliferation. PloS One 1:e128.

Park, G.H., H.M. Song and J.B. Jeong. 2016a. The coffee diterpene kahweol suppresses the cell proliferation by inducing cyclin D1 proteasomal degradation via ERK1/2, JNK and GKS3beta-dependent threonine-286 phosphorylation in human colorectal cancer cells. Food Chem. Toxicol. 95:142-148.

Park, G.H., J.H. Sung, H.M. Song and J.B. Jeong. 2016b. Anti-cancer activity of Psoralea fructus through the down-

regulation of cyclin D1 and CDK4 in human colorectal cancer cells. BMC Complement. Altern. Med. 16:373.

Park, G.H., S.C. Hong and J.B. Jeong. 2016c. Anticancer activity of the safflower seeds (*Carthamus tinctorius* L.) through inducing cyclin D1 proteasomal degradation in human colorectal cancer cells. Korean J. Plant Res. 29(3): 297-304.

Russell, A., M.A. Thompson, J. Hendley, L. Trute, J. Armes and D. Germain. 1999. Cyclin D1 and D3 associate with the SCF complex and are coordinately elevated in breast cancer. Oncogene 18:1983-1991.

Siegel, R.L., K.D. Miller, S.A. Fedewa, D.J. Ahnen, R.G.S. Meester, A. Barzi and A. Jemal. 2017. Colorectal cancer statistics, 2017. CA Cancer J. Clin. 67:177-193.

Thoms, H.C., M.G. Dunlop and L.A. Stark. 2007. p38-mediated inactivation of cyclin D1/cyclin-dependent kinase 4 stimulates nucleolar translocation of RelA and apoptosis in colorectal cancer cells. Cancer Res. 67:1660-1669.

Wang, Y., M. Deng, S.Y. Zhang, Z.K. Zhou and W.X. Tian. 2008. *Parasitic loranthus* from Loranthaceae rather than Viscaceae potently inhibits fatty acid synthase and reduces body weight in mice. J. Ethnopharmacol. 118:473-478.

Wen, C.C., L.F. Shyur, J.T. Jan, P.H. Liang, C.J. Kuo, P. Arulselvan, J.B. Wu, S.C. Kuo and N.S. Yang. 2011. Traditional Chinese medicine herbal extracts of *Cibotium barometz*, *Gentiana scabra*, *Dioscorea batatas*, *Cassia tora*, and *Taxillus chinensis* inhibit SARS-CoV replication. J. Trad. Complement. Med. 1:41-50.

Woo, I.S. and Y.H. Jung. 2017. Metronomic chemotherapy in metastatic colorectal cancer. Cancer Lett. 400:319-324.

Zhang, L., S.R. Koyyalamudi, S.C. Jeong, N. Reddy, T. Bailey and T. Longvah. 2013. Immunomodulatory activities of polysaccharides isolated from *Taxillus chinensis* and *Uncaria rhyncophylla*. Carbohydr. Polym. 98:1458-1465.

Zhang, L., A.S. Ravipati, S.R. Koyyalamudi, S.C. Jeong, N. Reddy, P.T. Smith, J. Bartlett, K. Shanmugam, G. Munch and M.J. Wu. 2011. Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. J. Agric. Food Chem. 59:12361-12367.

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