Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Adenovirus-mediated overexpression FADD induces a significant antitumor effect on human colorectal cancer cells both *in vitro* and *in vivo*

Xueling He, Xu Peng, Yan Liu, Xiaomei Zhang, Hualing Li, Hailin Yin*

Laboratory Animal Center of Sichuan University, Chengdu 610041, China

Correspondence to: yin37143@163.com

Received November 13, 2017; **Accepted** February 23, 2018; **Published** May 15, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.6.6

Abstract: The Wnt/β-catenin signaling pathway plays important roles in cancers such as colorectal cancer. Colon cancer cells secrete and express high levels of β-catenin, which may stimulate autocrine signaling and further enhance activities of the canonical Wnt signaling pathway. Free β-catenin in the cytoplasm and nucleus leads to its association with T cell factor (TCF)/lymphocyte enhancing factor (Lef) transcription factors, and subsequent transcriptional activation of downstream target genes. FADD plays a key role in cellular apoptosis in many different types of cancer. Therefore, a recombinant adenovirus is constructed, in which an apoptosis gene FADD is placed under control of a promoter containing Tcf-responsive elements. It is observed that FADD overexpression can suppress cell growth and enhance apoptosis of SW480 cells *in vitro*. In addition, Ad-FADD can also suppress the growth of subcutaneous xenografts in the nude mice. Together, these results suggest that Ad-FADD has anti-proliferative and pro-apoptotic effects in colon cancer cells, which provides a novel strategy for treatment of colorectal cancer.

Key words: Wnt/β-catenin; Fas-associated death domain protein (FADD); Apoptosis; Colon cancer.

Introduction

Colorectal cancer is the second and third most common cancer in women and men, respectively, accounting for approximately 10% of all cancer-related deaths. Previous studies have reported that the Wnt/β-catenin signaling pathway is mutated in about 90% of sporadic and hereditary colorectal cancers (1). Mutations result in the stabilization of β-catenin and its increased nuclear translocation to induce target gene expression (2). Some studies have reported that colon cancer stem cells secrete and express high β-catenin transcription activity, which may stimulate autocrine signaling and further enhance the activity of canonical Wnt signaling pathway (3, 4). β-catenin is a key activator of the canonical Wnt signaling pathway, which results in the stabilization and increase of β-catenin expression, and its translocation into the nucleus, where it binds to TCF/LEF proteins and provides a transcription activation domain such that targets genes can be activated.

Lately, though a few small molecules and biological agents that target the Wnt/β-catenin signaling have been identified, which targets in the pathway may offer an ideal therapeutic strategy is still remains unclear (5-7). Especially, Wnt/β-catenin signaling also plays important roles in in embryogenesis, tissue homeostasis, and regeneration; thus, once Wnt/β-catenin signaling is successfully targetted in cancer, it will require a fine balancing act. Beyond that, several known targets in the Wnt/β-catenin pathway are also involved in other pathways. As such, it remains unclear whether these potential therapeutic agents that target the Wnt/β-catenin pathway will be efficacious in combatting cancer and will even bring new physiological problems.

Transcription of downstream target genes is activated by the nuclear TCF/β-catenin complex, which results in tumorigenesis. These arise from hyperactivation of the classical Wnt classical signaling pathway (8, 9). As such, targeting the β-catenin/TCF complex and various downstream target genes for antitumor therapy may potentially be a novel strategy. Fas-associated protein with death domain (FADD), which is a classical adaptor protein in the apoptotic pathway, is the key adaptor protein that transmits apoptotic signals mediated by death receptors (DRs). FADD recently has been amplificated from many different types of cancer and has been linked to cancer progression (10-13).

In the present study, a recombinant adenovirus containing an apoptotic gene FADD is under control of a promoter containing Tcf-responsive elements, and can selectively and efficiently kill colon cancer cells with a hyperactivated β-catenin/Tcf pathway. This approach may provide a conceptual proof that aberrantly activated Wnt/β-catenin/Tcf pathways can be used to selectively target colon cancers.

Materials and Methods

Cell Culture

NIH 3T3 and SW480 cell lines were cultured in DMEM with high glucose (Thermo Scientific) supplemented with 10% fetal bovine serum (FBS; Invitrogen/GIBCO) and 1% of penicillin/streptomycin (Thermo Scientific). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The medium was replaced twice a week, and adherent cells were passaged

at 70%–80% confluence.

Viral production and transduction efficiency

Construction and production of adenovirus carrying the FADD gene (Ad-FADD) have recently been described (14). Prior to commencing the experiments, transduction efficiency for all cell lines to be used were determined using an adenoviral vector carrying the green fluorescent protein (GFP). The Ad-GFP or Ad-GFP-FAD was co-culture with SW480 cells and more than 80% of cells were transduced at 10^9TCID50/ml (data not shown). Therefore, in all subsequent experiments, cells were treated with 10^9TCID50/ml.

RNA Isolation and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Cells were harvested after infection with Ad- FADD for 72 hours. Afterwards, total RNA was extracted using the TRIzol solution (Invitrogen). 3 μg of RNA was used for the first-strand cDNA synthesis using SuperScript III (Thermo). 1μL of cDNA was then used to perform PCR using primers for FADD, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a control. The FADD gene was amplified by PCR with primer sequences 5'- acacagagaaggagaacgca -3' and 5'-gaaccaaagtccaggctgtg-3', while the GAPDH gene was amplified with primer sequences 5'-ccaaggagtaagacccctgg GA-3' and 5'-tggttgagcacagggtactt-3' as the internal standard. PCR conditions were as follows: denaturation at 94°C for 3 minutes, followed by 30 cycles of 95°C for 30s, 40°C for 30s, 72°C for 1min, and then a final extension at 72°C for 10 minutes. PCR products were run on a 2% agarose gel containing Gold View DNA dye.

Cell viability assay

The viability of cells at 48 and 72 hours after transfection was evaluated using a 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma, St Louis, MO, USA). MTT (at a concentration of 500 mg/ml) was added to the culture medium in each well of a 96-well plate, which was incubated for 4 hours at 37°C. Acidic isopropyl alcohol (0.04 N HCl/isopropyl alcohol) was immediately added to all wells and mixed vigorously such that the dark blue crystals dissolved effectively. Absorbance readings were recorded at 570 nm.

Nuclear Double Staining with Hoechst 33342/PI

Apoptosis of cells at 72 and 96 hours after transfection was evaluated using a Hoechst 33342/PI Detection Kit (Solarbio, China) as described in a previous study (15). The cells were washed three times with PBS (pH 7.4) and stained with Hoechst 33342/PI solution (1:1, V/V) for 20 minutes at 37°C in the dark. Cellular morphology was then observed by fluorescent microscopy, and approximately 100 cells from five random microscopic fields were counted.

Animals and treatments

Twenty 6-week-old female athymic BALB/c nude mice (18±2 g) were purchased from the Laboratory Animal Center of Sichuan University (China). Mice were inoculated subcutaneously with SW480 colorectal cancer cells suspension (2×10^6 cells/ml) 200μl. After 12 days, when most of the tumor volume reached 40 mm^3 in size, mice were randomly divided into the following treatment groups (with five mice per group): PBS, Ad, 10^9TCID50, and 10^{10}TCID50 groups. All mice were injected via the tail vein once every 4 days for six injections. The tumor size was measured by a Vernier calliper every 4 days. The tumor volume (mm^3) was calculated as (length×$width^2$)/2. At the end of the experiment, mice were sacrificed, and the tumors were separated, weighed, and sectioned for histological evaluation with hematoxylin and eosin (H&E) staining. The experimental protocols for all animal experiments were approved by the Ethics Committee of Sichuan University and were in accordance with the "Principles of Laboratory Animal Care" of the National Institutes of Health.

In situ TUNEL Assay

Apoptotic cells were labelled *in situ* using a Terminal deoxynucleotidyl transferase mediated dUTP Nick End Labeling (TUNEL) assay kit (Roche), in accordance with the manufacturer's protocol. Paraffin sections were deparaffinized and incubated in proteinase K (40 μg/ml) diluted in phosphate buffered saline (PBS, 0.1 M, pH 7.4) for 20 minutes at 37°C. Endogenous peroxidase was then quenched by incubation in 3.0% hydrogen peroxide for five minutes. The ends of fragmented DNA from tissues were labelled with terminal deoxynucleotidyl transferase (TdT) in the presence of digoxigenin-conjugated nucleotides and unlabeled nucleotides for 75 minutes at 37°C.

Deparaffinized slides were then incubated with anti-digoxigenin antibodies for 30 minutes, and these antibodies were detected by immunohistochemistry through exposure to a chromogenic substrate for 15 minutes. All sections were then counterstained with Harris' Haematoxylin. This was followed by dehydration in alcohol, a xylene rinse, and mounting onto a coverslip.

Statistical analyses

All data analyses involved estimation of the mean and SD using the SPSS software pachage, version 15.0 (SPSS, Inc., Chicago, IL, USA). Group means were compared through an one-way analysis of variance. The significance of the difference between groups was evaluated with Dunnett's multiple comparisons test. In this study, $p<0.05$ (two-tailed) was used as the level of statistical significance.

Results

Overexpression of FADD mediated by an adenovirus vector in SW480 cells

To examine the overexpression of FADD as mediated by Ad vectors in SW480 cells, the transfection rate was observed at 72 hours after treatment with Ad-FADD or Ad. As shown in Fig. 1A, more than 90% of SW480 cells expressed a green fluorescent signal. In addition, total RNA was isolated from cells infected with Ad-FADD or Ad and were subjected to RT-PCR. The cells infected with Ad-FADD expressed much higher FADD mRNA than those infected with Ad as a control. These results indicated that Ad-FADD infection resulted in a significant overexpression of FADD (Fig. 1B).

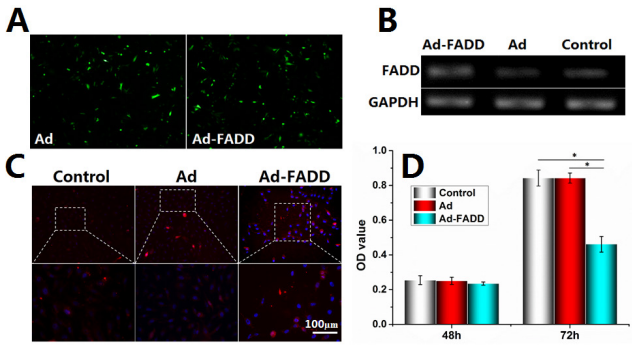

Figure 1. Ad-TCF/FADD antitumor effect on human colorectal cancer cells *in vitro.* Transfection rate of SW480 cells was observed at 72 h after treating with Ad and Ad-TCF/FADD. (B) The expression level of FADD in SW480 cells was measured at 72 h after transfecting with Ad and Ad- TCF/FADD. (C) SW480 cells apoptosis were observed by Hoechst/PI at 72 h after transfecting with Ad and Ad- TCF/FADD. (D) The effect of Ad- TCF/FADD on the proliferation of SW480 cells. Statistical significance: (*) $p < 0.01$.

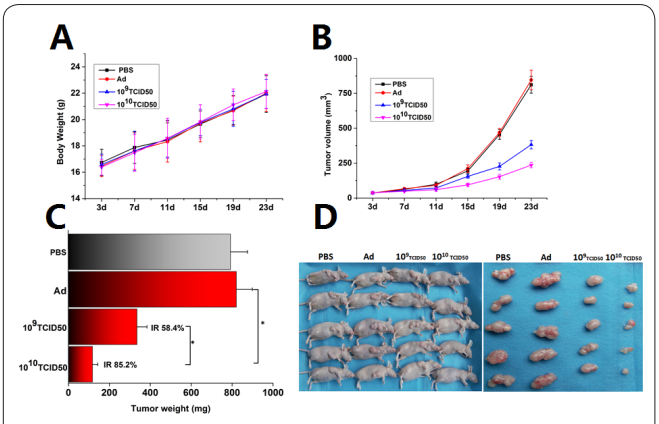

Figure 2. Ad-TCF/FADD antitumor effect on human colorectal cancer cell *in vivo.* (A, B) Changes in body weight and tumor volume after treatment with Ad- TCF/FADD, Ad, and PBS. (C) Tumor weights were measured after excision and plotted as the average tumor weight, and Tumor growth inhibition rates (IRs) were calculated. (D) Representative images of tumors separated from animals after 23 days. Statistical significance: (*) $p < 0.01$.

Effects of Ad-FADD on the proliferation of SW480 cells

To quantitatively examine the HCCS1-induced cytotoxicity, SW480 colorectal cancer cells and normal cell lines (NIH 3T3) were infected with Ad-FADD and Ad at 10^9TCID50, and cell viability was analyzed at 48 and 72 hours after infection. As shown in Fig. 1D, the proliferation of SW480 cells was clearly inhibited after infection with Ad-FADD, compared with Ad infection and control (treated with PBS) cells ($p<0.01$). However, no significant inhibition of Ad-FADD on proliferation was observed for NIH 3T3 cells (data not shown). These results suggest that SW480 cells are more sensitive to FADD-induced inhibition of proliferation.

Effect of Ad-FADD on the apoptosis of SW480 cells

To determine whether the reduced viability in SW480 cells was due to apoptosis, the cells were double stained with Hoechst 33342/PI, and the resulting nuclear morphological changes were observed under a fluorescence microscope. In control (treated with PBS) and Ad infection cells, the nuclei appeared to be round and even in shape, with intact chromatin that homogenously trasnmitted a faint blue fluorescence (Fig.1C). In contrast, after infection with Ad-FADD for 72 hours, cells showed increased intensity of blue fluorescence, nuclear shrinkage and chromatin condensation that are typical of early apoptosis. Dual-stained cells were not found. These results indicated that apoptosis indeed occurred in SW480 cells following infection with Ad-FADD.

Suppression of tumor growth in the nude mice by Ad-FADD

To investigate the antitumor effect of FADD *in vivo*, SW480 solid tumors were established in nude mice; after which, PBS, Ad, 10^9TCID50 and 10^{10}TCID50 Ad-FADD were injected via the tail vein once every 4 days for a total of six injections, and the therapeutic effect was monitored with tumor growth curves. The tumor showed a significant growth delay after administration of 10^9TCID50 or 10^{10}TCID50 Ad-FADD compared with administration of Ad or PBS ($p<0.01$), with the therapeutic effect occurring in a dose-dependent manner (Figs. 2B, 2C, 2D). On the other hand, the tumor development in Ad-treated mice was similar to PBS-treated mice ($p>0.05$). All mice were sacrificed, tumors were excised and and weighed at the study endpoint. As shown in Fig. 2C, the average weight of tumors in the 10^9TCID50 and 10^{10}TCID50 groups was significantly lighter than that in the Ad or PBS groups ($p<0.01$), while no difference was observed between the two latter groups ($p>0.05$). The inhibition rates by Ad-FADD reached 58.4% (109TCID50) and 85.2% (1010TCID50), respectively. However, significant side-effects of Ad-FADD on the body weight and major organs were not found.

Ad-FADD decreases proliferation and increases apoptosis of SW480 tumors *in vivo*

To investigate the effects of Ad-FADD on the histology of SW480 xenografts, tumor sections taken from mice were stained with H&E. As shown in Fig. 3B, more cancer cells with greater nuclear fragmentation was observed in H&E stained sections of 10^9TCID50 or 10^{10}TCID50 mice, compared to those in the Ad or

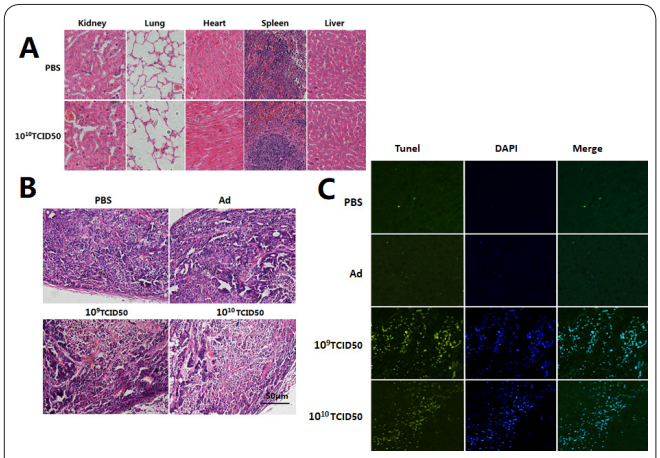

Figure 3. Ad-TCF/FADD antitumor effect on apoptosis of human colorectal cancer cell *in vivo.*(A) The effect of Ad- TCF/FADD on the major organs of mice. (B) Hematoxylin and Eosine (H&E) stained tumor tissue images after therapy. (C) TUNEL stained tumor tissue images for cell apoptosis.

PBS groups. To investigate the apoptotic effects of Ad-FADD in tumors, sections were stained for DNA fragmentation with the TUNEL assay. In comparison to the Ad or PBS groups, a clear increase in the level of apoptotic cells was observed in 10^9TCID50 and 10^{10}TCID50 groups (Fig. 3C). These findings suggest that Ad-FADD promoted apoptosis, which when combined with a reduction in the proliferation rate of tumor cells, was responsible for the dramatic inhibition of tumor growth *in vivo*.

Discussion

At present, surgery, chemotherapy, and radiotherapy are the main treatment of the tumor. Although the progress of these therapies has improved the cure rate of malignant tumors, there is still no breakthrough in the treatment of metastatic advanced cancer patients (17). Furthermore, radiotherapy and chemotherapy have serious harm to patients. Therefore, it is an urgent need to find a tumor treatment method for patients with minor damage and effective control of metastatic.

As a new therapeutic method, gene therapy has opened up a new way for the treatment of human disease (18). To choose a suitable carrier to ensure the safe and efficient introduction of the target cells to the target cells is one of the most critical problems in gene therapy. The vectors available include viral and non-viral vectors. Although non-viral vector has the advantages of safety and simple preparation, the efficiency of transfection is low and the time of gene expression is short, which affects its application. Adenovirus is the most widely used vehicle for gene therapy, especially for tumor gene therapy because of its high transfection efficiency and high expression efficiency. In recent years, with the further research of the molecular structure and infection mechanism of adenovirus, the improvement of the original adenovirus vector has achieved excellent results (19).

Recent studies have found that aberrant Wnt/β-catenin signaling pathway plays a key role in several cancers including colorectal cancer (20). Dysregulation of β-catenin signaling is thought to play an important role in early stages of human sporadic colorectal carcinogenesis (21). Several drugs have been investigated for their ability to inhibit the aberrantly activated Wnt/β-catenin signaling in colorectal cancers (22). β-catenin is involved in the Wnt signaling pathway to transactivate T cell factor (Tcf)/lymphocyte enhancing factor (Lef) transcription factors in the nucleus. Some of the genes activated by β-catenin/Tcf signaling include c-jun, c-myc, fibronectin, cyclin D1 and fra-1 (23-25). Free β-catenin in the cytoplasm and nucleus leads to its association with T cell factor (TCF)/lymphocyte enhancing factor (Lef) transcription factors, and to subsequent transcriptional activation of downstream target genes.

In this study, a recombinant adenovirus was constructed, in which an apoptotic gene FADD is placed under control of a promoter containing Tcf-responsive elements (26). The adenovirus was then transfected into a human colorectal cancer cell line SW480, which induced its overexpression.

We found that FADD overexpression could suppress the growth of cells by MTT assay and enhance apoptosis by Hoechst/PI staining. These results are in agreement with previous reports (26, 27). Lastly, the therapeutic effects of ad-FADD were assayed *in vivo*. The results showed that ad-FADD can significantly suppress the growth of subcutaneous xenografts in the nude mice, with inhibition rates reaching 58.4% (10^9TCID50) and 85.2% (10^{10}TCID50), respectively. H&E staining and TUNEL assays showed that colorectal cells underwent significant apoptosis with Ad-FADD treatment. Cancer formation is often a multistep process, which may include point mutations, deregulation or deletion of proto-oncogenes and anti-oncogenes, which altogether may be responsible for the development of cancer (28). The main advantage of gene therapy is the transfer of a particular gene to a specific group of mammalian or tumor cells such that the desired effect will be localized, and normal cells can be spared (29). In this study, no significant inhibition of Ad-FADD on proliferation was observed for NIH 3T3 cells *in vitro* (data not shown). In addition, no significant inhibition of Ad-FADD on body growth was also observed in the nude mice, nor were there were any significant side-effects of Ad-FADD observed on normal cells and major organs, both *in vivo* and *in vitro* (Figs. 2A, 3A).

In conclusion, we demonstrate that Ad-FADD has anti-proliferative and pro-apoptotic effects in colon cancer cells both *in vivo* and *in vitro*. These results provide a novel strategy to improve the therapeutic efficacy of other treatments in colorectal cancer patients with reduced side effects. Therefore, this study maybe provides a basis for further exploration of the roles of the FADD gene in the apoptotic regulation of colorectal cancer and other cancers with β-catenin abnormal expression.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by grants from the Nature Science Foundation of China (grant no.51573112) and the platform project of Sichuan Provincial Natural Science Foundation of China (grant no. 2016KJT0045-2014T).

References

1. Shim A R, Dong G Z, Lee H J, et al. Atractylochromene Is a Repressor of Wnt/β-Catenin Signaling in Colon Cancer Cells. 2015, 23(1):26-30.

2. Kobayashi M, Honma T, Matsuda Y, et al. Nuclear translocation of beta-catenin in colorectal cancer. British Journal of Cancer, 2000, 82(10):1689-93.

3. Kolligs F T, Bommer G, Göke B. Wnt/Beta-Catenin/Tcf Signaling: A Critical Pathway in Gastrointestinal Tumorigenesis. Digestion, 2002, 66(3):131-144.

4. Morin P. beta-catenin signaling and cancer.. Bioessays News & Reviews in Molecular Cellular & Developmental Biology, 1999, 21(12):1021-30.

5. Xu M, Liu X, Xu Y, et al. Co-expression of Axin and APC gene fragments inhibits colorectal cancer cell growth via regulation of the Wnt signaling pathway.. Molecular Medicine Reports, 2017.

6. Liu L J, Xie S X, Chen Y T, et al. Aberrant regulation of Wnt signaling in hepatocellular carcinoma. World Journal of Gastroenterology, 2016, 22(33):7486-7499.

7. Picco G, Petti C, Centonze A, et al. Loss of AXIN1 drives ac-

quired resistance to WNT pathway blockade in colorectal cancer cells carrying RSPO3 fusions. Embo Molecular Medicine, 2017, 9(3):293.
8. Tetsu O, Mccormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells.. Nature, 1999, 398(6726):422.
9. He T C, Sparks A B, Rago C, et al. Identification of c-MYC as a Target of the APC Pathway . Science, 1998, 281(5382):1509-1512.
10. Wang Q R, Yao X Q, Wen G, et al. Apigenin suppresses the growth of colorectal cancer xenografts via phosphorylation and up-regulated FADD expression. Oncology Letters, 2011, 2(1):43.
11. Yang Y W, Zhang C M, Huang X J, et al. Tumor-targeted delivery of a C-terminally truncated FADD (N-FADD) significantly suppresses the B16F10 melanoma via enhancing apoptosis. Sci Rep, 2016, 6:34178.
12. Lee E W, Seo J, Jeong M, et al. The roles of FADD in extrinsic apoptosis and necroptosis. Bmb Reports, 2012, 45(9):496-508.
13. Bowman BM, Sebolt KA, Hoff BA, et al. Phosphorylation of FADD by the kinase CK1alpha promotes KRASG12D-induced lung cancer. Sci Signal. 2015;8: ra9.
14. Chen R H, Mccormick F. Selective targeting to the hyperactive beta-catenin/T-cell factor pathway in colon cancer cells.. Cancer Research, 2001, 61(11):4445-9.
15. Chan C K, Supriady H, Goh B H, et al. Elephantopus scaber, induces apoptosis through ROS-dependent mitochondrial signaling pathway in HCT116 human colorectal carcinoma cells. Journal of Ethnopharmacology, 2015, 168:291-304.
16. Mateoiu C, Pirici A, Bogdan F. Immunohistochemical nuclear staining for p53, PCNA, Ki-67 and bcl-2 in different histologic variants of basal cell carcinoma. Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie, 2011, 52(1 Suppl):315-9.
17. Mituś J W, Walasek T, Reinfuss M, et al. Effectiveness of phyllodes tumor recurrences treatment - The role of surgery, radiotherapy and chemotherapy. Onkologia I Radioterapia, 2013, 22(1):59-64.
18. Ferrantini M, Belardelli F. Gene therapy of cancer with interferon: lessons from tumor models and perspectives for clinical applications.. Seminars in Cancer Biology, 2000, 10(2):145-57.
19. Zhou M, Peng J Q, Guo Y. Application of Different Vectors in Gene Therapy of Myocardial Ischemia. Progress in Modern Biomedicine, 2011.
20. Tai W P, Hu P J, Wu J, et al. The inhibition of Wnt/β-catenin signaling pathway in human colon cancer cells by sulindac.. Tumori, 2014, 100(1):97.
21. Kobayashi M, Honma T, Matsuda Y, et al. Nuclear translocation of beta-catenin in colorectal cancer. British Journal of Cancer, 2000, 82(10):1689-93.
22. Shan B E, Wang M X, Li R Q. Quercetin inhibit human SW480 colon cancer growth in association with inhibition of cyclin D1 and survivin expression through Wnt/β-catenin signaling pathway.. Cancer Investigation, 2009, 27(6):604.
23. He T C, Sparks A B, Rago C, et al. Identification of c-MYC as a Target of the APC Pathway. Science, 1998, 281(5382):1509-1512.
24. Tetsu O, Mccormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells.. Nature, 1999, 398(6726):422.
25. Soriano S, Kang D E, Fu M, et al. Presenilin 1 Negatively Regulates β-Catenin/T Cell Factor/Lymphoid Enhancer Factor-1 Signaling Independently of β-Amyloid Precursor Protein and Notch Processing. Journal of Cell Biology, 2001, 152(4):785-94.
26. Yin A, Jiang Y, Zhang X, et al. Overexpression of FADD enhances 5-fluorouracil-induced apoptosis in colorectal adenocarcinoma cells. Medical Oncology, 2010, 27(2):397-405.
27. Micheau O, Solary E, Hammann A, et al. Fas ligand-independent, FADD-mediated activation of the Fas death pathway by anticancer drugs. Journal of Biological Chemistry, 1999, 274(12):7987.
28. Astrin S M. Are there molecular targets for therapy of colon cancer?. Oncology, 1991, 5(10):127.
29. Sabaawy H E, Farley T, Ahmed T, et al. Synergetic effects of retrovirus IFN-alpha gene transfer and 5-FU on apoptosis of colon cancer cells.. Acta Haematologica, 1999, 101(2):82.