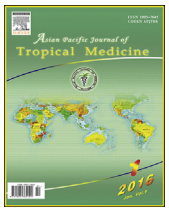
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2015.12.012

Mechanism of Wnt/β-catenin signaling pathway in enhanced malignant phenotype of non-small cell lung cancer induced by anti-angiogenesis therapy

Xiao-Xue Zhang[1], Ling-Ling Zhang[2], Huan-Lian Yang[2], Xiu-Wen Wang[3*]

[1]*Shandong University School of Medicine, Qingdao, Shandong 250000, China*

[2]*Department of Oncology, Binzhou People's Hospital, Binzhou, Shandong 256610, China*

[3]*Department of Oncology, Qilu Hospital of Shandong University, Ji'nan, Shandong 250000, China*

ARTICLE INFO

Article history:
Received 15 Oct 2015
Received in revised form 20 Nov 2015
Accepted 3 Dec 2015
Available online 19 Dec 2015

Keywords:
Anti-angiogenesis
Wnt1
β-Catenin
Epithelial mesenchymal transition
Proliferation and invasion

ABSTRACT

Objective: To study the mechanism of Wnt/β-catenin signaling pathway in the enhanced malignant phenotype of A549 cells of human non-small cell lung cancer induced by the anti-angiogenesis therapy.

Methods: The siRNA technique was employed to inhibit the expression of vascular endothelial growth factor (VEGF) in A549 cells and simulate the clinical course of anti-angiogenesis therapy. Real-time PCR and western blot were used to study the change in the expression of Wnt/β-catenin signaling molecules at the mRNA and protein level respectively, as well as the effect on the epithelial mesenchymal transition in A549 cells. The proliferation and invasion abilities of tumor cells were detected to discuss the mechanism of Wnt/β-catenin signaling pathway in the enhanced malignant phenotype of non-small cell lung cancer induced by the anti-angiogenesis therapy.

Results: The specific siRNA could significantly inhibit the expression of VEGF in cells to simulate the anti-angiogenesis therapy. Under the action of 50 nM VEGF siRNA, the proliferation ability of A549 significantly increased ($P < 0.05$). After being treated with VEGF siRNA, the invasion ability of cells increased. Twenty-four hours after the transcription of 50 nM siRNA into cells, the number of cells that come through the membrane was 278.3 ± 12.9. Compared with the Ctrl siRNA group, when VEGF was inhibited, the expression of *β-catenin* and *Cyclin D1* increased by 86% and 55% respectively. Meanwhile, the expression of E-cadherin decreased, while the one of vimentin increased.

Conclusions: siRNA can significantly inhibit the expression of VEGF. For the anti-angiogenesis therapy, the inhibited expression of VEGF can activate the Wnt/β-catenin signaling pathway to cause the epithelial mesenchymal transition and then the enhanced malignant phenotype of non-small cell lung cancer.

1. Introduction

The metabolism of tumor cells is more active than that of the normal cells, especially the malignant cancer. The new blood vessels can provide more oxygen and nutrients for the tumor cells to offer the pathway for the hematogenous metastasis of tumor cells. Meanwhile, the endothelial cells of new blood vessels can express and secrete many growth factors, such as the insulin-like growth factor and platelet-derived growth factor, which can stimulate the growth of adjacent tumor cells [1–4]. Therefore, the angiogenesis plays a key role in the infiltration and metabasis of tumor growth. The angiogenesis that can inhibit the tumor would lead to the insufficient nutrients for the growth of tumor and thus cause the inhibition against the growth of tumor cells [5]. In recent years, the research on the anti-angiogenesis therapy of tumor has been a hot topic in the field of tumor biotherapy and a great number of chemical small molecule drugs and monoclonal antibody agents for the anti-angiogenesis have been sold on the market [6–8]. These drugs mainly affect the angiogenesis in the tumor for the therapy. However, the previous researches reported that these drugs might reduce the efficiency of some other anti-cancer drugs and enhance the invasion of tumors. For instance, it's found that the angiogenesis inhibitor could reduce the delivery of cytotoxic drugs to the tumor location and thus affect the therapeutic effect of drugs [9,10]. With

*Corresponding author: Xiu-Wen Wang, PhD, Department of Chemotherapy, Qilu Hospital of Shandong University, No.107, West Wenhua Road, Ji'nan, Shandong 250000, China.
Tel: +86 18560082906, +86 13791122979
E-mail: sdmzc0518@163.com

Peer review under responsibility of Hainan Medical College.
Foundation project: It was supported by National Natural Science Foundation (No. 81372530).

1995-7645/Copyright © 2016 Hainan Medical College. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

the in-depth research and development of anti-angiogenesis drugs, more and more research data indicated that these treatments had the certain limitations [11,12].

In this study, by using siRNA to inhibit the expression of vascular endothelial growth factor (VEGF) in A549 cells, it was to study the effect of change in the expression of VEGF on Wnt/β-catenin signaling pathway. Besides, by analyzing the correlation between the change in the proliferation and invasion of A549 cells and the expression of VEGF, it was to discuss the mechanism of Wnt/β-catenin signaling pathway in the enhanced malignant phenotype of non-small cell lung cancer induced by anti-angiogenesis therapy.

2. Materials and methods

2.1. Main materials and reagents

The human non-small cell lung cancer A549 cells were purchased from Committee on Type Culture Collection of Chinese Academy of Sciences and the cells conserved in the liquid nitrogen in this laboratory.

VEGF siRNA was purchased from Santa Cruz Biotechnology (America, item No. sc-29520); the total RNA extraction kit from TIANGEN Biotech (China, item No. DP430); miRNA isolation kit from mirVana (America, item No. AM1561); the reverse transcription kit from Applied Biosystems (America, item No. 4366597); Real-time PCR fluorescent quantitative kit from Bio-Rad (America, item No. 172–5264); ReadyPrep protein extraction kit from Bio-Rad (America); BCA protein quantitative kit from Vazyme Biotech (China, item No. E112-01); Gsk3β, p-Gsk3β and VEGF monoclonal antibodies from Santa Cruz Biotechnology (America, item No. 377213, 81496 and 390741); Transwell chamber from Corning (America, item No. 4395); Matrigel from BD (America); horseradish peroxidase-labeled secondary antibody from Beijing Zhongshan Jinqiao Biotechnology; ECL Chemiluminescent Substrate Reagent Kit from Life Technologies (America, item No. WP20005).

CO_2 cell culture incubator (Thermo Scientific Series 8000), DNA/RNA analyzer (Qubit Fluorometer); the cell nuclear transfer system (Amaxa Nucleofector) and the fluorescent quantitative PCR system (Bio-Rad-CFX96 Touch) were also used in this study.

2.2. Methods

2.2.1. Cell culture and transfection

A594 cells were conserved in the liquid nitrogen. DMEM culture medium was used after the recovery of cells; MRC-5 was cultured in EMEM culture medium that contained 15% fetal bovine serum (GIBIC) at 37 °C and 5% CO_2.

The cell nuclear transfer system of Amaxa Nucleofector was used for transfection of siRNA. By regulating and optimizing the electroporation parameters, the exogenous siRNA was then transfected into the cell nucleus directly.

To verify the inhibition efficiency of siRNA and confirm its concentration range, A549 cells were seeded on 6-well plate. When cells grown up by about 70%, the cell nuclear transfer system of Amaxa Nucleofector was employed to transfect VEGF siRNA and Control siRNA with the different concentrations. Real-time PCR and western blot were adopted to detect the inhibition efficiency of siRNA.

2.2.2. Real-time PCR

The collected cells were washed with PBS (RNase free). miRNA isolation kit and total RNA extraction kit were used to extract miRNAs and total RNA. Qubit Fluorometer system was used to detect the concentration and purity of RNA. The total RNA was reversely transcripted to cDNA following the instruction manual of reverse transcription kit (it was guaranteed that only the mature miRNAs were transcripted, but no precursor reaction on miRNA. The transcription product cDNA was used as the template). The Real-time PCR was employed to detect the expression of related genes. The mRNA sequence of *E-cadherin*, *Vimentin*, *β-catenin* and *Cyclin D1* genes could be referred to NCBI database and then the Real-time PCR primers could be designed.All primers were synthesized by Shanghai Generay Biotech Co, Ltd.

2.2.3. Cell invasion assay

To study the effect of inhibited expression of VEGF on the growth of A549 cells, A549 cells were transfected with 0, 5, 10 and 50 nM VEGF siRNA respectively, while the control was Ctrl siRNA with the random sequence. MTT method was employed to detect the effect of inhibited expression of VEGF on the proliferation of A549 cells. Matrigel was taken out and then melted at 4 °C over night. The pre-cooled serum-free DMEM medium was used to dilute Matrigel to the final concentration of 1 mg/mL 100 μL digested Matrigel was added in the center of bottom of upper chamber of Transwell and then it was incubated at 37 °C to be gelatinous. 200 μL DMEM medium was added in each well for the reconstruction.

After being trypsinized and centrifuged, the culture medium was removed and cells were washed with sterile PBS. The serum-free culture medium was used to resuspend the cells. A549 cells was added in the upper chamber of Transwell, while the lower chamber contained DEME medium with 10% FBS for the further 24 h of culture. The cells were cultured at 37 °C. After the culture, the liquid in the upper chamber was removed. Then the upper chamber was taken out and the cells that were not transferred were wiped by the cotton swab. 4% Paraformaldehyde was used for the fixation at room temperature for 10 min and it was then stained with the crystal violet. The number of membrane-penetrating cells in 3 fields was counted as mean ± SD under the inverted optical microscope and the statistical analysis was performed.

2.2.4. Western blotting

The collected cells were washed with PBS twice. After being trypsinized and centrifuged, the supernatant was removed. ReadyPrep protein extraction kit was used to extract the total protein (After being put on the ice for 30 min, the probe-type ultrasound was used to produce the short impact with the appropriate frequency on the ice. The lysis mixture was centrifuged at 4 °C and 13 000 r/min for 20 min). The supernatant was transferred to the new centrifuge tube. BCA kit was employed to detect the protein concentration.

SDS-PAGE electrophoresis was performed on 20 μg protein samples. The gel was soaked in the transfer buffer for 10 min of equilibrium. It was installed with the transfer 'sandwich' with 100 V and 45–60 min. After the transfer, PVDF film was washed with TBS for 10–15 min. The film was placed in TBS/T blocking buffer containing 5% (w/v) skimmed milk powder and shaken at the room temperature for 1 h. Then the primary antibody with the appropriate degree of dilution was added [diluted with TBST containing 1% (w/v) skimmed milk powder]. It was incubated at the room temperature for 2 h and then the film was washed with TBST for 3 times, 5–10 min every time. The film was incubated with the secondary antibody (1:10 000, horseradish peroxidase-labeled) that was diluted with

TBST containing 0.05% (w/v) skimmed milk powder. It was incubated at the room temperature for 1 h and then the film was washed with TBST for 3 times, 5–10 min every time. It was exposed and then photographed to save the experimental results. The experiment was repeated three times. Quantity one v4.62 was used to measure the gray value of molecular band (trace tracking). The semi-quantitative value of target protein/reference protein was chosen as the quantitative basis and the statistical analysis was performed.

2.2.5. Statistical analysis

The experimental data was treated with SPSS11.5. The results were expressed by mean ± SD. The *t* test was employed for the comparison between groups, where $P < 0.05$ indicated the significant difference.

3. Results

3.1. Inhibition effect of siRNA on expression of VEGF

Results showed that the specific siRNA could significantly inhibit the expression of VEGF in cells, especially the concentration of siRNA over 50 nM ($P < 0.05$). The western blot was used to further detect the change in the protein level. The results also showed that the concentration of siRNA over 50 nM could significantly inhibit the expression of VEGF.

3.2. Effect of inhibited expression of VEGF on proliferation and invasion of A549 cells

The results showed that, under the action of 50 nM VEGF siRNA, the proliferation of A549 was significantly increased ($P < 0.05$) (Table 1). The cell invasion assay was performed to study the effect of inhibited expression of VEGF on the invasion of A549 cells. Table 2 showed the number of cells that transferred through Transwell chamber. According to the results, the inhibited expression of VEGF could enhance the invasion of A549 cells. The number of cells that transferred through the basement membrane in the control group was 154.7 ± 8.3. After being treated with VEGF siRNA, the invasion of cell was enhanced. Twenty-four hours after the transfection of 50 nM siRNA, the number of cells that transferred through the membrane was 278.3 ± 12.9.

Table 1
OD of A549 cells treated with different concentrations of siRNA.

Time (h)	siRNA			
	0 nm	5 nm	10 nm	50 nm
0	0.79 ± 0.04			
24	1.06 ± 0.02	0.91 ± 0.03	1.04 ± 0.03*	1.36 ± 0.01*
36	0.98 ± 0.04	0.86 ± 0.09	0.93 ± 0.03*	1.27 ± 0.01**
48	0.83 ± 0.06	0.76 ± 0.06	0.79 ± 0.06	0.93 ± 0.01*

*$P < 0.05$, **$P < 0.01$ *vs.* control group.

3.3. Effect of inhibited expression of VEGF on Wnt/β-catenin signaling pathway

According to the results, when A549 were treated with 50 nM VEGF siRNA, after inhibiting the expression of VEGF, the expression of *β-catenin* and *Cyclin D1* was significantly increased in cells. Compared with the Ctrl siRNA group, after inhibiting VEGF (50 nM, 24 h), the expression of *β-catenin* and *Cyclin D1* was increased by 86% and 55% respectively, which indicated that after inhibiting VEGF, Wnt/β-catenin signaling pathway was activated. Meanwhile, the results of western blot showed the limited change in the total amount of Gsk3β, while p-Gsk3β was inversely proportional to the expression of VEGF.

To specify the effect of inhibited expression of VEGF on epithelial mesenchymal transition (EMT) process of A549 cells, the change in the expression of *E-cadherin* and *Vimentin* at mRNA level was detected. The results showed that, after inhibiting VEGF, the activation of Wnt/β-catenin signaling pathway caused EMT process. It could detect that after inhibiting VEGF, the expression of *E-cadherin* was down-regulated, while the expression of *Vimentin* was up-regulated.

4. Discussion

The research on the anti-angiogenesis therapy of tumor has been the hot topic in the field of tumor biotherapy and a great number of chemical small molecule drugs and monoclonal antibody agents for the anti-angiogenesis have been sold on the market [6–8], which mainly affected the angiogenesis of tumors for the treatment of cancer. The anti-angiogenesis therapy has also been the part of clinical standard therapy of non-small cell lung cancer. However, the further researches on the mechanism of anti-angiogenesis showed that these drugs might reduce the efficiency of some other anti-cancer drugs and enhance the malignant phenotype of tumors [9].

One of the important characteristics of tumor cells is the abnormal metabolism. To maintain the energetic metabolism, the tumor cells require more oxygen and nutrients. Thus the growth of tumors will depend on the angiogenesis to guarantee the sufficient oxygen and nutrients. Accordingly, in the clinical practice, the anti-angiogenesis drugs or the combination with the common cytotoxic drugs are adopted to treat some kind of tumors. To be specific, the tumor cells can be 'starved to death' to realize the inhibition against the growth of tumors. Presently, the research on the anti-angiogenesis therapy of tumor has been the hot topic in the field of tumor biotherapy and a great number of chemical small molecule drugs and monoclonal antibody agents

Table 2
Result of cell invasion assay.

Field of vision	Control group	siRNA			
		0 nm	5 nm	10 nm	50 nm
V1	152	142	169	204	269
V2	148	177	176	214	273
V3	164	169	153	226	293
Total	154.7 ± 8.3	162.7 ± 18.3	166.0 ± 11.8	214.7 ± 11.0*	278.3 ± 12.9**

Compared with the control group, *$P < 0.05$, **$P < 0.01$.

for the anti-angiogenesis have been sold on the market. However, the molecular mechanism of these combination therapies has not been clear and even more and more researches have indicated the limitations of these therapies. According to Dutch scholar Van der Veldt, it's found that the angiogenesis inhibitor could reduce the delivery of cytotoxic drugs to the tumor location and thus affect the therapeutic effect of drugs [13]. While Conley *et al.* also found that the tumor could accumulate the cells with the strong ability of invasion to adapt to the anti-angiogenesis therapy [11].

VEGF is the specific heparin-binding growth factor of vascular endothelial cell, which can induce the angiogenesis inside the body. It is the most effective factor to stimulate the vasculogenesis and angiogenesis, which plays a key role in the development of tumor angiogenesis [12–14]. At present, most anti-angiogenesis drugs or monoclonal antibody drugs achieve the goal of anti-angiogenesis by inhibiting the expression of main target VEGF [15–17]. Wnt/β-catenin signaling pathway is of critical importance to regulate the proliferation, differentiation and metabasis of cells. In normal mature cells, the Wnt signal is in the inhibitory state. In case of the abnormal activation of Wnt signaling pathway, there would be different kinds of diseases such as the tumor [18,19]. The abnormal activation of Wnt signaling pathway has been proved in the lung cancer. The previous researches indicated that Wnt/β-catenin signaling pathway might promote the expression of *Cyclin D1* [20,21], where Cyclin D1 is the key regulatory protein in G_1 phase of cell cycle and also the classical downstream gene of Wnt/β-catenin signaling pathway. The regulation of Wnt/β-catenin signaling pathway on VEGF has been clear. According to the previous researches, in the colon cancer cells, there is the binding element of TCF-4 at 805 bp of VEGF promoter, which means that Wnt/β-catenin signaling pathway can up-regulate the expression of VEGF.

Based upon above findings, it's believed that the anti-angiogenesis therapy can inhibit the angiogenesis of tumor tissues and has also some limitations. Considering that Wnt/β-catenin signaling pathway can regulate the expression of VEGF at multiple levels and Wnt/β-catenin signaling pathway has also been proved with the abnormal activation in many kinds of tumors [22–25], this paper studied the mechanism of Wnt/β-catenin signaling pathway in the enhanced malignant phenotype of non-small cell lung cancer induced by the anti-angiogenesis therapy at the cellular level, in order to provide some experimental reference for the research, development and optimization of anti-angiogenesis drugs.

By specifically inhibiting the expression of VEGF in A549 cells using siRNA, it was to achieve the simulation environment of anti-angiogenesis drugs. The experimental results showed that 50 nM siRNA could significantly inhibit the expression of VEGF ($P < 0.01$). After inhibiting the expression of VEGF, we analyzed the correlation between the expression of key molecules of *β-catenin* and *Cyclin D1* of Wnt/β-catenin signaling pathway and the inhibited expression of VEGF. The results showed that, after inhibiting VEGF, the expression of *β-catenin* and *Cyclin D1* was all significantly increased. Compared with the Ctrl siRNA group (siRNA with the random sequence), the expression of *β-catenin* and *Cyclin D1* was increased by 86% and 55% respectively, which indicated that Wnt/β-catenin signaling pathway was activated. Meanwhile, the western blot was employed to detect the key molecule of Gsk3β and the results showed the limited change in the total amount of Gsk3β after inhibiting VEGF, while p-Gsk3β was inversely proportional to the expression of VEGF. In Wnt/β-catenin signaling pathway, the phosphorylated Gsk3β could not further degrade the β-catenin. To be specific, Wnt/β-catenin signaling pathway was in the active state to cause the accumulation of β-catenin and then the metabasis into the nucleus. By analyzing the correlation between the change in the proliferation and invasion of A549 cells and the expression of VEGF, it's found that the inhibited expression of VEGF would lead to the certain up-regulation of proliferation and invasion abilities of A549 cells. Because the invasion ability of cells is closely related to EMT process, the EMT in the tumor cells to make them possess the invasion and metabasis abilities is the important process in the development of tumor. When Wnt/β-catenin signaling pathway is activated, β-catenin is bound with T-cell factor/lymphoid enhancer factor in the nucleus to regulate the expression of related target genes [26–28]. The previous researches also indicated the close correlation between the expression of *Vimentin* and *β-catenin* in the nucleus [29,30]. According to the experimental results, the activation of Wnt/β-catenin signaling pathway caused EMT. It could be detected that after inhibiting VEGF, the expression of *E-cadherin* was down-regulated, while the expression of *Vimentin* was up-regulated. Accordingly, it's believed that there may be some kind of compensation mechanism. In other words, when the expression of VEGF is inhibited, the important upstream Wnt/β-catenin signaling pathway is activated to simulate the expression of VEGF. But the activation of Wnt/β-catenin signaling pathway will enhance the series of malignant phenotypes of tumor cells. The specific molecular mechanism will be the focus of further researches. Meanwhile, in our opinion, it's necessary to evaluate the benefits and limitations of anti-angiogenesis therapy. Such therapy may be improved by regulating the administration order of cytotoxic drugs and anti-angiogenesis drugs or using the combination therapy with other drugs that make use of targeting progenitor cell signaling.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Novakova V, Sandhu GS, Dragomir-Daescu D, Klabusay M. Apelinergic system in endothelial cells and its role in angiogenesis in myocardial ischemia. *Vasc Pharmacol* 2015; http://dx.doi.org/10.1016/j.vph.2015.08.005. S1537–1891(15)00181-0.

[2] Zhao Y, Adjei AA. Targeting angiogenesis in cancer therapy: moving beyond vascular endothelial growth factor. *Oncologist* 2015; **20**(6): 660-673.

[3] Masri B, van den Berghe L, Sorli C, Knibiehler B, Audigier Y. Apelin signalisation and vascular physiopathology. *J Soc Biol* 2009; **203**(2): 171-179.

[4] Tycinska AM, Lisowska A, Musial WJ, Sobkowicz B. Apelin in acute myocardial infarction and heart failure induced by ischemia. *Clin Chim Acta* 2012; **413**(3–4): 406-410.

[5] Abéngozar MA, de Frutos S, Ferreiro S, Soriano J, Perez-Martinez M, Olmeda D, et al. Blocking ephrinB2 with highly specific antibodies inhibits angiogenesis, lymphangiogenesis, and tumor growth. *Blood* 2012; **119**(19): 4565-4576.

[6] Hurwitz HI, Douglas PS, Middleton JP, Sledge GW, Johnson DH, Reardon DA, et al. Analysis of early hypertension and clinical outcome with bevacizumab: results from seven phase III studies. *Oncologist* 2013; **18**(3): 273-280.

[7] Manso L, Moreno F, Márquez R, Castelo B, Arcediano A, Arroyo M, et al. Use of bevacizumab as a first-line treatment for metastatic breast cancer. *Curr Oncol* 2015; **22**(2): e51-e60.
[8] Wagner AD, Thomssen C, Haerting J, Unverzagt S. Vascular-endothelial-growth-factor (VEGF) targeting therapies for endocrine refractory or resistant metastatic breast cancer. *Cochrane Database Syst Rev* 2012; **7**: CD008941.
[9] Casanovas O. Cancer: limitations of therapies exposed. *Nature* 2012; **484**(7392): 44-46.
[10] Van der Veldt AA, Lubberink M, Bahce I, Walraven M, de Boer MP, Greuter HN, et al. Rapid decrease in delivery of chemotherapy to tumors after anti-VEGF therapy: implications for scheduling of anti-angiogenic drugs. *Cancer Cell* 2012; **21**(1): 82-91.
[11] Conley SJ, Gheordunescu E, Kakarala P, Newman B, Korkaya H, Heath AN, et al. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. *Proc Natl Acad Sci U S A* 2012; **109**(8): 2784-2789.
[12] Kerbel RS, Guerin E, Francia G, Xu P, Lee CR, Ebos JM, et al. Preclinical recapitulation of antiangiogenic drug clinical efficacies using models of early or late stage breast cancer metastatis. *Breast* 2013; **22**(Suppl. 2): S57-S65.
[13] Kaur S, Chang T, Singh SP, Lim L, Mannan P, Garfield SH, et al. CD47 signaling regulates the immunosuppressive activity of VEGF in T cells. *J Immunol* 2014; **193**(8): 3914-3924.
[14] Diaz R, Nguewa PA, Redrado M, Manrique I, Calvo A. Sunitinib reduces tumor hypoxia and angiogenesis, and radiosensitizes prostate cancer stem-like cells. *Prostate* 2015; **75**(11): 1137-1149.
[15] Eswarappa SM, Fox PL. Antiangiogenic VEGF-Ax: a new participant in tumor angiogenesis. *Cancer Res* 2015; **75**(14): 2765-2769.
[16] Papadimitriou K, Rolfo C, Dewaele E, Van De Wiel M, Van den Brande J, Altintas S, et al. Incorporating anti-VEGF pathway therapy as a continuum of care in metastatic colorectal cancer. *Curr Treat Options Oncol* 2015; **16**(4): 18.
[17] Park do J, Thomas NJ, Yoon C, Yoon SS. Vascular endothelial growth factor a inhibition in gastric cancer. *Gastric Cancer* 2015; **18**(1): 33-42.
[18] Nelson WJ, Nusse R. Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* 2004; **303**(5663): 1483-1487.
[19] Jansson L, Kim GS, Cheng AG. Making sense of Wnt signaling-linking hair cell regeneration to development. *Front Cell Neurosci* 2015; **9**: 66.
[20] Wang H, Wang H, Makki MS, Wen J, Dai Y, Shi Q, et al. Overexpression of β-catenin and cyclinD1 predicts a poor prognosis in ovarian serous carcinomas. *Int J Clin Exp Pathol* 2013; **7**(1): 264-271.
[21] Li ZL, Shao SH, Jiao F, Yue Z, Ma Y. Cyclin D1 regulates lung cancer invasion and metastasis. *Sheng Li Xue Bao* 2012; **64**(1): 55-61.
[22] Watcharasit P, Suntararuks S, Visitnonthachai D, Thiantanawat A, Satayavivad J. β-Catenin involvement in arsenite-induced VEGF expression in neuroblastoma SH-SY5Y cells. *Environ Toxicol* 2014; **29**(6): 672-678.
[23] Watcharasit P, Visitnonthachai D, Suntararuks S, Thiantanawat A, Satayavivad J. Low arsenite concentrations induce cell proliferation via activation of VEGF signaling in human neuroblastoma SH-SY5Y cells. *Environ Toxicol Pharmacol* 2012; **33**(1): 53-59.
[24] Mottet D, Dumont V, Deccache Y, Demazy C, Ninane N, Raes M, et al. Regulation of hypoxia-inducible factor-1alpha protein level during hypoxic conditions by the phosphatidylinositol 3-kinase/Akt/glycogen synthase kinase 3beta pathway in HepG2 cells. *J Biol Chem* 2003; **278**(33): 31277-31285.
[25] Hwang I, Kim J, Jeong S. β-Catenin and peroxisome proliferator-activated receptor-δ coordinate dynamic chromatin loops for the transcription of vascular endothelial growth factor A gene in colon cancer cells. *J Biol Chem* 2012; **287**(49): 41364-41373.
[26] Fernández JG, Rodríguez DA, Valenzuela M, Calderon C, Urzúa U, Munroe D, et al. Survivin expression promotes VEGF-induced tumor angiogenesis via PI3K/Akt enhanced β-catenin/Tcf-Lef dependent transcription. *Mol Cancer* 2014; **13**: 209.
[27] Torres VA, Tapia JC, Rodriguez DA, Lladser A, Arredondo C, Leyton L, et al. E-cadherin is required for caveolin-1-mediated down-regulation of the inhibitor of apoptosis protein survivin via reduced beta-catenin-Tcf/Lef-dependent transcription. *Mol Cell Biol* 2007; **27**(21): 7703-7717.
[28] Abeyrathna P, Su Y. The critical role of Akt in cardiovascular function. *Vasc Pharmacol* 2015; **74**: 38-48.
[29] Prasad CP, Mirza S, Sharma G, Prashad R, DattaGupta S, Rath G, et al. Epigenetic alterations of CDH1 and APC genes: relationship with activation of Wnt/beta-catenin pathway in invasive ductal carcinoma of breast. *Life Sci* 2008; **83**(9–10): 318-325.
[30] Serman L, Nikuseva MT, Serman A, Vranic S. Epigenetic alterations of the Wnt signaling pathway in cancer: a mini review. *Bosn J Basic Med Sci* 2014; **14**(4): 191-194.