Int J Clin Exp Med 2016;9(5):8812-8820
www.ijcem.com /ISSN:1940-5901/IJCEM0020184

*Original Article*

# The mechanism of myocardial differentiation and angiogenesis regulated by Notch/Dll4 and VEGF interactions

Fei Liang[1], Shi-Qing Cheng[2], Bo Jin[3], Zhi-Ju Tian[4], Cheng-Wei Zou[1], Zhi-Ming Jiang[5]

*Departments of [1]Cardiac Surgery, [2]Clinical Laboratory, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250021, Shandong, China; [3]Department of Thoracic Surgery, Zhang Qiu People's Hospital, Zhangqiu 250200, Shandong, China; [4]Department of Thoracic Surgery, Zhang Qiu People's Hospital, Zhangqiu, Jinan 250200, China; [5]Department of Critical-Care Medicine, Shandong Provincial Qian Foshan Hospital Affiliated to Shandong University, Jinan 250014, Shandong, China*

Received November 20, 2015; Accepted April 15, 2016; Epub May 15, 2016; Published May 30, 2016

**Abstract:** Objective: Notch/Dll4 pathway is involved in regulating cell proliferation, apoptosis and differentiation *etc*. The vascular endothelial growth factor (VEGF) pathway has been identified as a major pathway in regulation of angiogenesis. This study investigated the mechanism of regulation of angiogenesis and myocardial differentiation by Notch/Dll4 and VEGF pathways. Methods: Mesenchymal stem cells (MSCs) were obtained from rat bone marrow under aseptic conditions and verified using the flow cytometry (FCM). Notch/Dll4 and VEGF plasmids were transfected into MSCs. Real-time PCR was used to analyze myocardial differentiation related genes including GATA-4, α-actin, cTnI and Cx43 expression in MSCs before and after transfection in order to evaluate the effects of Notch/Dll4 and VEGF interactions on myocardial differentiation. MSCs were co-transfected with Notch/Dll4 and VEGF plasmids. Real-time PCR and Western blot were used to measure endothelial cells specific markers including CD31, vWF, and Tie-2 expression both in mRNA and protein levels. In addition, this study analyzed the effects of Notch/Dll4 and VEGF interactions on angiogenesis ability using *in vitro* angiogenesis assay kit. Results: Overexpression of Dll4 in MSCs by cDNA transfection significantly inhibited GATA-4 and cTnI expression ($P < 0.01$), while overexpression of VEGF promoted GATA-4 and cTnI expression ($P < 0.05$). Co-transfection of Dll4 and VEGF plasmids or transfection of VEGF plasmid alone resulted in Cx43 gene expression significantly increase ($P < 0.05$). In addition, overexpression of Dll4 inhibited the expression of vWF and Tie-2, while overexpression of VEGF, endothelial promoted CD31, vWF, and Tie-2 to increase. Conclusion: Notch/Dll4 suppressed the differentiation process of MSCs to myocardial cells, while VEGF promoted the process. Notch/Dll4 and VEGF interactions regulated the process of differentiation and angiogenesis of MSCs.

**Keywords:** Myocardial differentiation, angiogenesis, Notch/Dll4, vascular endothelial growth factor (VEGF)

## Introduction

As an evolutionarily conserved intercellular pathway, the Notch pathway plays an important role in many biological process including cellular differentiation, proliferation, and apoptosis [1, 2]. Recently, the Notch pathway, particularly vascular-specific delta-like ligand 4 (Dll4) signaling, has been identified as another pivotal pathway in regulation of vascular development [3-5]. The Notch receptors and ligands are membrane proteins across the membrane only once. Therefore Notch pathway is a relatively simple way of signal transduction to facilitate communication between adjacent cells, which doesn't need participation of the second messenger and phosphorylation. Signaling molecules were exhausted via protein hydrolysis. This type of signal transduction method cannot amplify signal, but is very important for accurate control [6].

The development of the heart is a complex cascade of regulatory process [7], which includes that stem cells differentiate to cardiac progenitor cells [8] which further migrate and differentiate to cardiomyocytes and related blood vessels develop and form, *etc* [9, 10]. The process of angiogenesis is dependent on comprehensive regulation to either inhibit or promote angiogenesis. The vascular endothelial growth factor (VEGF) is one of the most potent

angiogenic factors to promote angiogenesis. Notch/Dll4 signaling pathways can promote blood vessels maturation and control vessel sprouting and branching therefore to regulate excessive proliferation of blood vessels [11, 12]. Extensive studies have highlighted the relationship between Notch/Dll4 and VEGF pathways. It has been confirmed that Notch/Dll4 and VEGF pathways play an important role in regulating embryonic vascular development and tumor angiogenesis. It is through the coordinating role of Notch/Dll4 and VEGF pathways to guarantee the normal formation of new blood vessels [13, 14]. However, the interaction between Notch/Dll4 and VEGF, as well as the mechanism of this interaction in the regulation of cardiac differentiation and angiogenesis remain obscure. Therefore we conducted this study to determine whether Notch/Dll4 and VEGF interactions are associated with myocardial differentiation and angiogenesis and its possible mechanism.

## Materials and methods

### *Experimental animals*

4-week-old specific pathogen free (SPF) Sprague-Dawley rats (250±50 g, Grade I, purchased from Better Biological Engineering co., Ltd., Nanjing, Jiangsu, China) were housed in plastic cages and maintained under an artificial 12 h/12 h light/dark cycle at 18~25°C. The rats had *ad libitum* access to standard rat chow and tap water. All of the experiments were conducted in accordance with the European Communities Council Directive (2010/63/EU) for the use of experimental animals.

### *Experimental reagents and instruments*

Low glucose DMEM (purchased from Gibco, USA); FBS (purchased from Zhejiang Tianhang Biotechnology Co., Ltd., Hangzhou, Zhejiang, China); FITC-conjugated anti-rat CD29 and PE-conjugated anti-rat CD44 (purchased from eBioscience, USA); Transfection Reagent Lipofectamine 2000 (purchased from Invitrogen, USA); RNA extraction kit (purchased from QIAGEN, Germany); reverse transcription kit (purchased from Applied Biosystems, USA); SYBR Green real-time PCR Master Mix (purchased from Applied Biosystems, USA); ReadyPrep protein extraction kit (purchased from Bio-Rad, USA); BCA protein Assay Kit (purchased from Vazyme Biotech Co., Ltd., Nanjing, Jiangsu, China); CD31, vWF, Tie-2 and GAPDH monoclonal antibodies (purchased from Abcam, UK); horseradish peroxidase (HRP) labeled secondary antibody (purchased from Wuhan Boster Biological, Wuhan, Hubei, China); PVDF membrane (0.22 μm) (purchased from Millipore, USA); ECL chemiluminescence detection kit (purchased from Millipore, USA); *in vitro* angiogenesis assay kit (purchased from Biovision, USA). $CO_2$ incubator: SANYO; inverted microscope: Olympus; nucleic acid quantitative analysis instrument: Qubit Fluorometer; fluorescence quantitative PCR detection system: Applied Biosystems 7500.

Culture and identification of rat bone marrow mesenchymal stem cells [15, 16].

12 SPF SD rats (4 wk) were sacrificed by cervical dislocation and their femurs and tibia were carefully flushed with low glucose DMEM to flush the marrow cavities using a 0.45-mm syringe needle until the bones become pale. After being centrifuged for 5 min at 1000 rpm, the cell sediment was resuspended in low glucose DMEM containing 10% FBS and cultivated in a 60 mm dish at 37°C in a 5% $CO_2$ humidified incubator. To isolate MSCs, after 48 hrs of culture, non-adherent cells were removed and adherent cells were maintained. On reaching 70%-80% confluence, these adherent cells were replanted. The medium was changed every 2-3 days.

The cultured MSCs were retrieved by trypsin-EDTA digestion. Cell aliquots ($1\times10^6$) were washed with cold PBS and resuspended in 100 μl of PBS per EP tube and stained with FITC-conjugated anti-rat CD29 and PE-conjugated anti-rat CD44 at a concentration of 2 μg/mL at 4°C for 30 min. One tube of unstained cells was prepared as a control for the antibodies. Cells were examined using a BD FACS calibur cytometer and analyzed using CellQuest software.

### *Construction of plasmid vector and cell transfection*

The eukaryotic plasmid vector pcDNA3.1 was used. Coding sequences of the target genes were inserted between BamH I and Xhol restriction sites in order to obtain Dll4 and VEGF plasmids. The construction of plasmid was conducted by Wuhan Transduction Biological Laboratory (Wuhan, Hubei, China).

**Table 1.** The primers used in Real-time PCR

| Gene | GenBank Accession | Primer (5'-3') | Length | Tm |
|---|---|---|---|---|
| GATA-4 | NM_001310610.1 | For: CTGTGCCAACTGCCAGACTA | 20 | 61.3 |
| | | Rev: AGATTCTTGGGCTTCCGTTT | 21 | 60.5 |
| α-actin | M12233.1 | For: AGCCATGTACGTAGCCATCC | 20 | 60.0 |
| | | Rev: CTCTCAGCTGTGGTGGTGAA | 20 | 60.2 |
| cTnI | NM_017144.2 | For: CCTGCGTGGCAAGTTTAA | 20 | 59.9 |
| | | Rev: TTCCTTCTCAATGTCCTCCT | 22 | 60.2 |
| Cx43 | NM_016975.3 | For: TCTCGCCTATGTCTCCTCC | 20 | 61.0 |
| | | Rev: TGTAGTTCGCCCAGTTTT | 21 | 61.2 |
| CD31 | NM_001305158.1 | For: CTCCTAAGAGCAAAGAGCAACTTC | 24 | 61.2 |
| | | Rev: TACACTGGTATTCCATGTCTCTGG | 24 | 61.6 |
| vWF | NM_011708 | For: CTCTTTGGGGACGACTTCATC | 21 | 60.1 |
| | | Rev: TCCCGAGAATGGAGAAGGAAC | 22 | 60.9 |
| Tie-2 | NM_001290551.1 | For: GCTGAGAACAACATAGGAT | 20 | 62.1 |
| | | Rev: CTGAGTTGAACTGAACAGC | 19 | 60.6 |
| GAPDH | NM_014364 | For: TGTGGGCATCAATGGATTTGG | 21 | 60.9 |
| | | Rev: ACACCATGTATTCCGGGTCAAT | 22 | 61.4 |

For, forward; Rev, reverse.

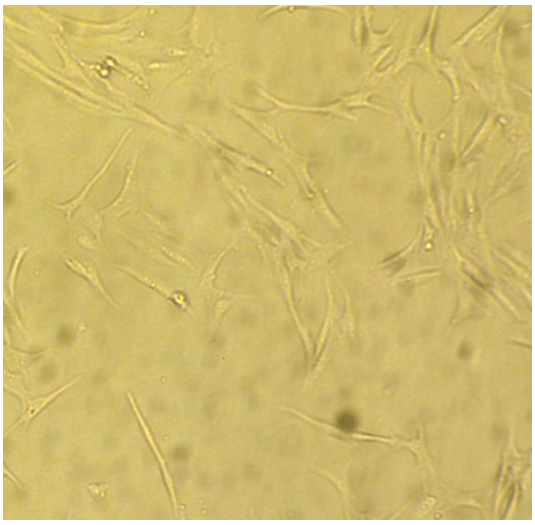

**Figure 1.** The 1st passage MSCs adherent to the wall after culture for 24 hrs (×400).

*Transfection of MSCs by lipofection*

According to Lipofectamine 2000 liposome transfection reagent instruction, the 4th passage MSCs were digested by pancreatic enzyme, and then was counted. It was inoculated at $3\times10^4$/mL to 6-well plate. On reaching 80% confluence, 1 μl Lipofectamine 2000 was diluted with 50 μl serum-free medium then mixed with diluted plasmids. The mixture was added to each well and incubated at 37°C and 5% $CO_2$ for 20 min. After 6 hrs, it was cultured in low glucose DMEM containing 10% FBS.

*Detection of expression of GATA-4, α-actin, cTnI, Cx43, CD31, vWF, and Tie-2 by RT-PCR*

Cell samples were washed with PBS (RNase free) and centrifuged to obtain cell sediments. Total RNA was extracted from cell sediments following the manufacturer's instructions. RNA samples' concentration and purity were measured using Qubit Fluorometer. Reverse transcription was undertaken using reverse transcription kit according to the manufacturer's protocol. The primer and probe sequences of the target genes and reference gene were obtained from the NCBI database. The construction of primers was conducted by Sangon Biotech Co., Ltd. (Shanghai, China). The specific sequences were shown in **Table 1**.

PCR was performed using the Applied Biosystems® 7500 Fluorescence Quantitative PCR Detection System in 96-well microtitre plates using a final volume of 25 μl. Amplifications were performed starting with a 2 min activation step at 50°C, 10 min temple denaturation at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min.

Relative expression levels were determined with the comparative threshold cycle (Ct) method, with amplification efficiency set at 97%.

*Detection of expression of CD31, vWF, and Tie-2 by Western blot*

The protein was extracted from cell samples using ReadyPrep protein extraction kit. Mixture was centrifuged at 4°C and 13000 r/min for 20 min. The supernatant was transferred into new centrifugal tube. Then BCA protein quantifying kit was employed to detect the concentration of

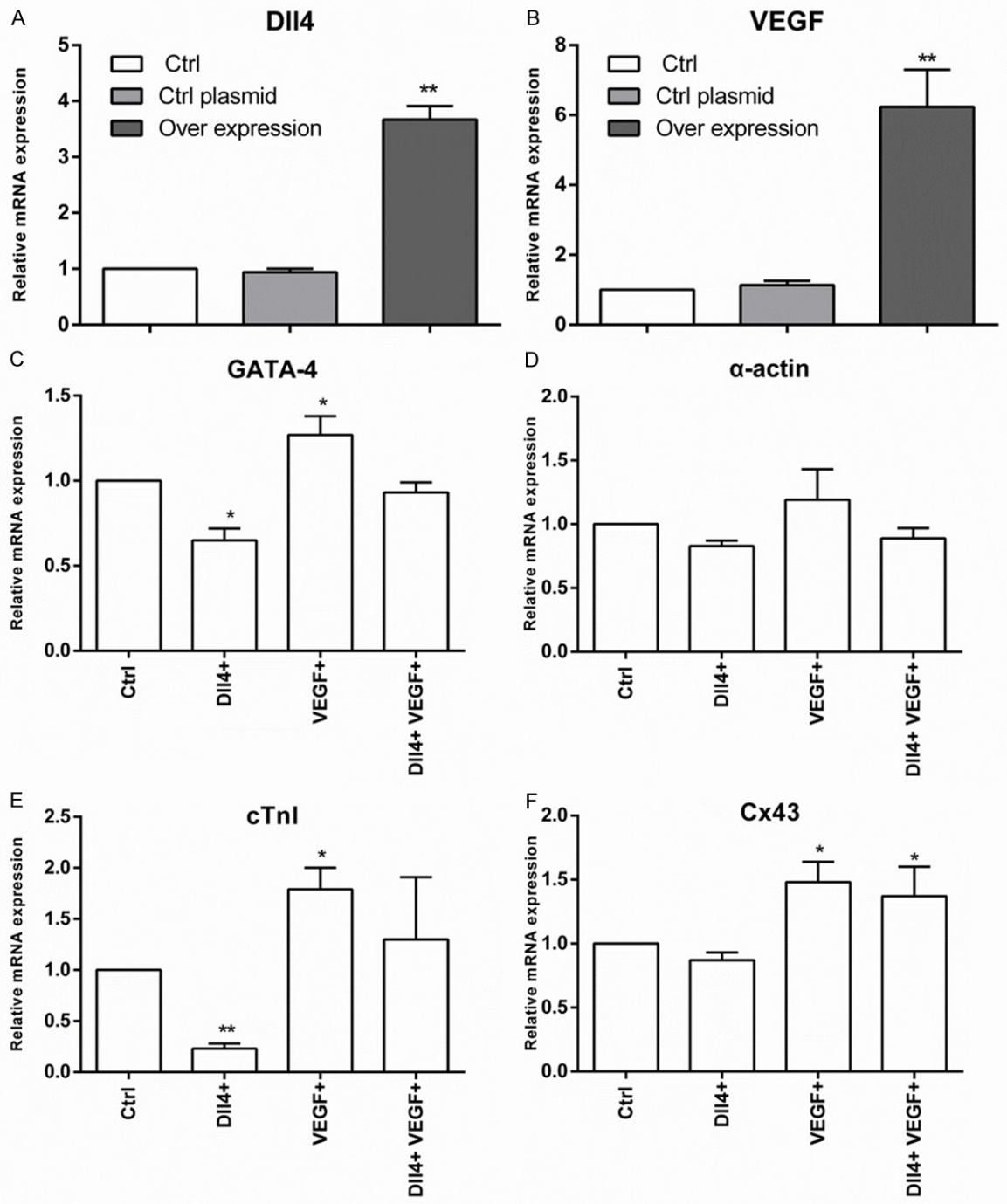


**Figure 2.** Myocardial differentiation related gene expression.

protein. Protein samples were given SDS-PAGE electrophoresis. They were transferred to polyvinylidene fluoride membrane using the semi-dry transfer buffer. After being blocked with 5% nonfat milk powder for 1 h, the primary antibodies which was diluted with TBST (CD31, vWF, and Tie-2) were added and then incubated at 4°C overnight. The membrane was washed with TBST for three times and then the HRP-labeled secondary antibodies were added and incubated in the room temperature for 1 h. Afterwards, it was washed with TBST for three times. Blots were visualized and quantified using Quantity one v4.62 software. All samples

were run in the same gel to minimize variability and the quantitative results for each protein were normalized to GAPDH.

*In vitro angiogenesis*

According to *in vitro* angiogenesis assay kit instructions, the matrigel at 50 μl/well was laid into 96-well plates to solidify to analyze the capillary-like tube formation of MSCs. MSCs were resuspended in DMEM medium containing 5% serum at $1\times10^4$/ml. 100 μl cell suspension was plated on matrigel at 37°C and 5% $CO_2$. After 12 hrs, tube images were obtained by using an inverted microscope.

*Statistical analysis*

Statistical analyzes were performed using statistical software SPSS 17.0. The data are expressed as mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls *post hoc* test for multiple comparisons. Values of $P < 0.05$ were considered statistically significant.

## Results

*Culture and identification of rat marrow mesenchymal stem cells*

After being cultured for 24 hrs, cells began to adhere to the wall and demonstrate fusiform morphology (**Figure 1**). After being cultured for 3 days, the cells were digested using trypsin and passed in the 1:2 ratio. The 3rd passage MSCs were measured for specific markers including CD29 and CD44 using FCM. The results showed that positive rates of CD29 and CD44 on MSCs were 98.4 % and 99.6% respectively indicating high purity of MSCs obtained.

*The effects of Notch/Dll4 and VEGF interaction on myocardial differentiation related gene expression*

MSCs were cultured for 3 passages then transfected with Dll4 and VEGF plasmids. Empty vector was used as control. 24 hrs after transfection, real-time PCR was used to measure Dll4 and VEGF overexpression. As shown in **Figure 2A**, **2B**, after being transfected with Dll4 and VEGF plasmids, Dll4 and VEGF gene expression were significantly increased ($P < 0.01$) compared to the control group.

As shown in **Figure 2C** and **2E**, overexpression of Dll4 inhibited expression of GATA-4 and cTnI, while overexpression of VEGF promoted it ($P < 0.05$). After co-transfection of Dll4 and VEGF, there was no difference of GATA-4 expression level between the Dll4+VEGF group and the control group. The overexpression of Dll4 and VEGF was not associated with α-actin expression (**Figure 2D**). As shown in **Figure 2F**, Co-transfected

Dll4 and VEGF or VEGF alone caused Cx43 regulation ($P < 0.05$).

*The effects of Notch/Dll4 and VEGF interactions on angiogenesis*

As shown in **Figure 3A**, after Dll4 overexpression, the change of CD31 expression was not significant, but the expression of vWF and Tie-2 were decreased by 23% and 39% respectively. However, after VEGF overexpression, CD31, vWF and Tie-2 were significantly increased ($P < 0.05$). Compared to the empty vector, CD31, vWF and Tie-2 expression levels were increased by 38%, 41%, and 29% respectively. The results of protein level were shown in **Figure 3B**, which were consistent with mRNA expression level, i.e. Dll4 over-expression can suppress the expression of vWF and Tie-2, while VEGF overexpression can promote the expression of endothelial cell-specific markers including CD31, vWF and Tie-2.

The changes of angiogenesis ability after Dll4 and VEGF overexpression were analyzed using *in vitro* angiogenesis assay kit. There was no capillary-like tube formed in the Dll4+ group. In the VEGF+ group, there were a lot of capillary-like tubes. The formation of capillary-like tubes was observed in the Dll4+VEGF+ group, but they were much less than VEGF+ group.

## Discussion

Notch pathway includes Notch receptors, Notch ligands, other Notch effectors, and regulatory molecules. Morgan [2] and his colleagues found Notch genes in the mutant flies for the first time. There were four types of Notch receptors (Notch1-4) and five types of Notch ligands (Delta-like 1, 3, 4, Jagged1, and Jagged2) in mammals. Notch signal was generated via adjacent cells Notch ligand-receptor interactions and went into the nucleus through the configuration change to bind with CSL and form

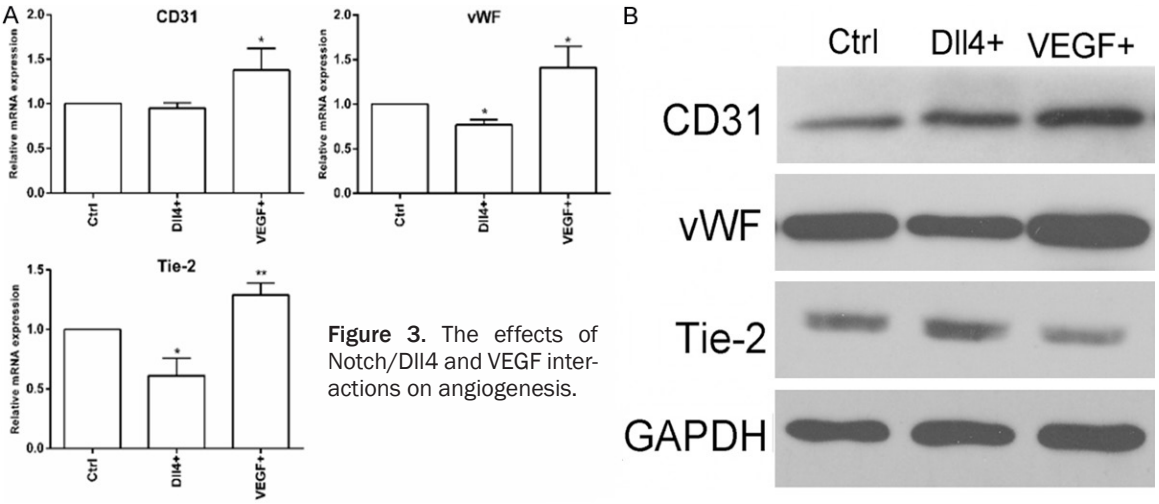


**Figure 3.** The effects of Notch/Dll4 and VEGF interactions on angiogenesis.

transcriptional activation complex exerting its biological effects. Notch signal transduction enables target cells directly receive signals from their adjacent cells, which does not require second messengers and protein kinase to amplify. It plays an important role in embryonic development, tissue homeostasis, and stem cell differentiation. A number of studies have found that Notch signaling pathway may regulate angiogenesis, which suggests that Notch signaling may be used as targets for the angiogenesis-targeting therapy [17, 18]. Notch signaling pathway regulates angiogenesis via four ligands (Dll1, Dll4, Jagged1, and Jagged2) and 3 receptors (Notch1, Notch3 and Notch4), wherein Dll4 is the main ligand binding with Notch to regulate the formation of new blood vessels [19]. In some hematological malignancies such as acute lymphoblastic leukemia and lymphoma, there were abnormal activation of the Notch signaling pathway found [20], suggesting that Notch signaling pathway plays an important regulatory role in endothelial cell proliferation and differentiation [21]. In terms of regulating stem cell differentiation, more in-depth studies are about the regulatory role of Notch signaling pathway in the process of embryonic stem cell differentiation into cardiomyocytes. The study found, Notch signaling pathway plays an important regulatory role [22-24] to the differentiation of cardiac cells in embryonic stem cells. The theory to use embryonic stem cells in order to induce cardiomyocytes regenerate, thus to treat cardiovascular disease has long been proposed [25]. However, the exact mechanism of induction and other issues have not been resolved yet.

VEGF is a very important factor to promote blood vessel growth, especially in tumor tissues. VEGF can participate in various physiological and pathological angiogenesis process via its receptors. In some solid tumors or hematological malignancies, there is abnormally high expression of VEGF detected, which is associated with unfavorable prognosis [26]. In recent years, VEGF, in addition to promote angiogenesis, were found to promote embryonic stem cells and adipose stem cells differentiate to myocardial cells [27, 28]. However the specific molecular mechanism is still unclear. Therefore, it is very important to study the mechanism of the role in stem cell differentiation in order to provide the theoretical basis for the treatment of cardiovascular diseases. Taylor [29] and his colleagues found that Notch/Dll4 activation can downregulate VEGFR2 expression. A variety of evidence has shown that VEGF and Dll4/Notch signaling pathway are related in the process of angiogenesis and the regulation of stem cell differentiation. However the specific mechanism of the interactions is still far from being clear. Therefore, we launched the study to investigate Notch/Dll4 and VEGF interactions regulating angiogenesis and myocardial differentiation and its related mechanism. We hope we can provide some theoretical basis for the treatment of cardiovascular diseases.

MSCs are self-renewing and pluripotent stem cells. MSCs can readily obtain immunogens and have low immune rejection. Therefore they are often used in stem cell differentiation experiments. We obtained MSCs from rat bone marrow and verified them according to stan-

dards by International Cell Therapy Association [30]. We used FCM to measure the CD29 and CD44 positive rates in obtained MSCs. The CD29 and CD44 positive rates reached 98.4% and 99.6% respectively indicating high purity of obtained MSCs.

In order to study the effects of Dll4 and VEGF on MSCs differentiation to myocardial cells, we transfected separately and co-transfected Dll4 and VEGF expression plasmids in order to obtain cells with over-expression of Dll4 and VEGF. In terms of the choice of plasmid, we chose pcDNA3.1 because pcDNA3.1 increased enzyme loci and CMV drive expression on the basis of pcDNA3.0. We measured myocardial differentiation related genes such as GATA-4, alpha actin, cTnI and Cx43 on mRNA level. The results showed that Dll4 and VEGF over-expression can cause GATA-4 downregulation and upregulation respectively ($P < 0.05$). After co-transfection of Dll4 and VEGF plasmids, the expression of GATA-4 has no significant difference compared to the control group.

α-actin is widely present in the myocardial cells thus considered specific structural protein of the myocardial cells. We found that there was no significant correlation between α-actin expression level and Dll4 and VEGF expression level. During experiment, after transfection of VEGF, α-actin appeared to increase, but the difference was not significant. The possible reasons could be that α-actin is usually present in the myocardial cells with high differentiation. The fact that α-actin significant changes were not detected may be due to the timing. Over-expression of Dll4 significantly inhibited cTnI expression ($P < 0.01$), while over-expression of VEGF promoted cTnI expression ($P < 0.05$). After co-transfection of Dll4 and VEGF plasmids, cTnI expression had no significant difference compared to the control group. Co-transfection of Dll4 and VEGF plasmids and separate transfection of VEGF plasmid can cause Cx43 gene expression increase ($P < 0.05$). The results suggest that Notch and VEGF play antagonistic roles against each other in the process of stem cells differentiation to myocardial cells. This coordinative effect maintains the normal differentiation process of stem cells.

We used the 5th generation of MSCs to conduct experiment which was consistent with the results from Wang [31] and her colleagues. The results showed that after over-expression of Dll4, the change of CD31 expression was not significant, while the expression of vWF and Tie-2 were decreased by 23% and 39% respectively. After transfection of VEGF expression plasmid, CD31, vWF and Tie-2 in MSCs were significantly increased ($P < 0.05$). Compared to the empty vector, CD31, vWF and Tie-2 expression levels were increased by 18%, 41%, and 29% respectively. The test result of protein level were basically consistent with the mRNA expression level, i.e. Dll4 over-expression can suppress the expression of vWF and Tie-2, while VEGF over-expression can promote the endothelial cell-specific markers including CD31, vWF and Tie-2. Further *in vitro* angiogenesis kit analyzed the change of angiogenesis ability after Dll4 and VEGF over-expression.

In conclusion, according to the above results, Notch activation may inhibit angiogenesis. But there are also reports having the exact opposite conclusion. In terms of interpretation of the opposite conclusions, we believe that it was due to different tissue sources and cell types. Depending on different types of tissues and cells, Notch/D114 signal pathway could play different roles to either promote or inhibit angiogenesis, which is consistent with the literature reported [32].

## Disclosure of conflict of interest

None.

**Address correspondence to:** Zhi-Ming Jiang, Department of Critical-Care Medicine, Shandong Provincial Qian Foshan Hospital Affiliated to Shandong University, No. 16766, Jingshi Road, Jinan 250014, Shandong, China. Tel: +86 531 89269292; Fax: +86 531 89269292; E-mail: jiang7708@sina.com

## References

[1] Palmer WH, Deng WM. Ligand-independent mechanisms of notch activity. Trends Cell Biol 2015; 25: 697-707.

[2] Colombo M, Galletti S, Garavelli S, Platonova N, Paoli A, Basile A, Taiana E, Neri A, Chiaramonte R. Notch signaling deregulation in multiple myeloma: A rational molecular target. Oncotarget 2015; 6: 26826-40.

[3] Li JL, Harris AL. Notch signaling from tumor cells: a new mechanism of angiogenesis. Cancer Cell 2005; 8: 1-3.

[4] Phng LK, Gerhardt H. Angiogenesis: a team effort coordinated by notch. Dev Cell 2009; 16: 196-208.
[5] Gale NW, Dominguez MG, Noguera I, Pan L, Hughes V, Valenzuela DM, Murphy AJ, Adams NC, Lin HC, Holash J, Thurston G, Yancopoulos GD. Haploinsufficiency of delta-like 4 ligand results in embryonic lethality due to major defects in arterial and vascular development. Proc Natl Acad Sci U S A 2004; 101: 15949-15954.
[6] Baron M. An overview of the Notch signalling pathway. Semin Cell Dev Biol 2003; 14: 113-119
[7] Cataliotti A, Tonne JM, Bellavia D, Martin FL, Oehler EA, Harders GE, Campbell JM, Peng KW, Russell SJ, Malatino LS, Burnett JC Jr, Ikeda Y. Long-term cardiac pro-B-type natriuretic peptide gene delivery prevents the development of hypertensive heart disease in spontaneously hypertensive rats. Circulation 2011; 123: 1297-1305.
[8] Mishra R, Vijayan K, Colletti EJ, Harrington DA, Matthiesen TS, Simpson D, Goh SK, Walker BL, Almeida-Porada G, Wang D, Backer CL, Dudley SC Jr, Wold LE, Kaushal S. Characterization and functionality of cardiac progenitor cells in congenital heart patients. Circulation 2011; 123: 364-373.
[9] Hu N, Han X, Lane EK, Gao F, Zhang Y, Ren J. Cardiac-specific overexpression of metallothionein rescues against cigarette smoking exposure-induced myocardial contractile and mitochondrial damage. PLoS One 2013; 8: e57151.
[10] Jiang XY. Nicotine inhibits myocardial differentiation and early heart development and its related mechanism research. PhD thesis, Southern Medical University 2011.
[11] Lobov IB, Cheung E, Wudali R, Cao J, Halasz G, Wei Y, Economides A, Lin HC, Papadopoulos N, Yancopoulos GD, Wiegand SJ. The Dll4/Notch pathway controls postangiogenic blood vessel remodeling and regression by modulating vasoconstriction and blood flow. Blood 2011; 117: 6728-6737.
[12] Zheng W, Tammela T, Yamamoto M, Anisimov A, Holopainen T, Kaijalainen S, Karpanen T, Lehti K, Ylä-Herttuala S, Alitalo K. Notch restricts lymphatic vessel sprouting induced by vascular endothelial growth factor. Blood 2011; 118: 1154-1162.
[13] Zhang JR, Ji CY. Notch/Dll4 pathway to promote angiogenesis induced by leukemia research. The 7th China Tumor Academic Conference 2012.
[14] Zhang J, Ye J, Ma D, Liu N, Wu H, Yu S, Sun X, Tse W, Ji C. Cross-talk between leukemic and endothelial cells promotes angiogenesis by VEGF activation of the Notch/Dll4 pathway. Carcinogenesis 2013; 34: 667-677.
[15] Yang L, Zhang RH, Xie HJ, et al. Establishment of a stable isolation culture system and identification of rat bone marrow mesenchymal stem cells. Journal of Clinical Rehabilitative Tissue Engineering Research 2009; 13: 1064-1068.
[16] Deng HY. miR-1 mediated Notch pathway regulating rat MSCs differentiation to myocardial cells. Master's Degree Thesis. Nanchang University 2012.
[17] Yan M, Plowman GD. Delta-like 4/Notch signaling and its therapeutic implications. Clin Cancer Res 2007; 13: 7243-6.
[18] Roca C, Adams RH. Regulation of vascular morphogenesis by Notch signaling. Genes Dev 2007; 21: 2511-24.
[19] Weng AP, Ferrando AA, Lee W, Morris JP 4th, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, Aster JC. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004; 306: 269-71.
[20] Zhu YH. Roles of VEGF and Dll4/Notch signaling pathways in angiogenesis and their relationship in patients with acute myeloid leukemia. Master Degree Thesis. Shandong University 2008.
[21] Shepler SA, Patel AN. Cardiac cell therapy: a treatment option for cardiomyopathy. Crit Care Nurs Q 2007; 30: 74-80.
[22] Hu JY, Xu HX, Tian YH, et al. Effects of Notch signaling pathway on the differentiation of embryonic stem cells to cardiomyocytes. Journal of Wuhan University 2013; 34: 485-485.
[23] Nemir M, Croquelois A, Pedrazzini T, Radtke F. Induction of cardiogenesis in embryonic stem cells via downregulation of Notch1 signaling. Circ Res 2006; 98: 1471-8.
[24] Klüppel M, Wrana JL. Turning it up a Notch: cross-talk between TGF beta and Notch signaling. Bioessays 2005; 27: 115-8.
[25] Shepler SA, Patel AN. Cardiac cell therapy: a treatment option for cardiomyopathy. Crit Care Nurs Q 2007; 30: 74-80.
[26] Ferrara N. Molecular and biological properties of vascular endothelial growth factor. J Mol Med (Berl) 1999; 77: 527-43.
[27] Chen Y, Amende I, Hampton TG, Yang Y, Ke Q, Min JY, Xiao YF, Morgan JP. Vascular endothelial growth factor promotes cardiomyocyte differentiation of embryonic stem cells. Am J Physiol Heart Circ Physiol 2006; 291: H1653-8.
[28] Song YH, Gehmert S, Sadat S, Pinkernell K, Bai X, Matthias N, Alt E. VEGF is critical for spontaneous differentiation of stem cells into cardiomyocytes. Biochem Biophys Res Commun 2007; 354: 999-1003.

[29] Taylor KL, Henderson AM, Hughes CC. Notch activation during endothelial cell network formation in vitro targets the basic HLH transcription factor HESR-1 and downregulates VEGFR-2/KDR expression. Microvasc Res 2002; 64: 372-83.

[30] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315-7.

[31] Wang LL. Local RAS induced hypoxia induced RAS secretion and angiogenesis in bone marrow mesenchymal cells. Master Degree Thesis. Nanjing Medical University 2013.

[32] Zhao XC. Regulation of tumor vessel structure and function by Notch pathway and the underlying mechanism. Ph.D. Dissertation. The 4th Military Medical University 2014.

[33] Zhang JR. The interaction and mechanism of Notch/Dll4 and VEGF signaling pathway in angiogenesis of acute myeloid leukemia. Ph.D. Dissertation. Shandong University 2013.