Biochemical and Biophysical Research Communications 457 (2015) 221–226

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Double-stranded RNA transcribed from vector-based oligodeoxynucleotide acts as transcription factor decoy

Xiao Xiao [a,1], Yi Gang [a,b,1], Honghong Wang [c], Jiayin Wang [d], Lina Zhao [e], Li Xu [a,*], Zhiguo Liu [a,*]

[a] State Key Laboratory of Cancer Biology and Xijing Hospital of Digestive Diseases, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, PR China
[b] Department of Infectious Diseases, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, Shaanxi Province, PR China
[c] No. 518 Hospital of Chinese People's Liberation Army, Xi'an 710043, Shaanxi Province, PR China
[d] The Genome Institute, Washington University in St. Louis, St. Louis, MO 63108, USA
[e] Department of Radiation Oncology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, PR China

ARTICLE INFO

Article history:
Received 2 December 2014
Available online 27 December 2014

Keywords:
Transcription factor decoy
VB-ODN
shRNA
NF-κB

ABSTRACT

In this study, we designed a short hairpin RNA vector-based oligodeoxynucleotide (VB-ODN) carrying transcription factor (TF) consensus sequence which could function as a decoy to block TF activity. Specifically, VB-ODN for Nuclear factor-κB (NF-κB) could inhibit cell viability and decrease downstream gene expression in HEK293 cells without affecting expression of NF-κB itself. The specific binding between VB-ODN produced double-stranded RNA and NF-κB was evidenced by electrophoretic mobility shift assay. Moreover, similar VB-ODNs designed for three other TFs also inhibit their downstream gene expression but not that of themselves. Our study provides a new design of decoy for blocking TF activity.

1. Introduction

Transcription factors (TFs) are well defined in regulating gene expression by binding to their consensus sequence/cis-elements leading to either transactivation or suppression of the target genes and therefore play very important roles in physiological and pathological processes [1]. Manipulation of TFs is often employed to study relevant downstream effects and transcription factor decoy (TFD) is a tool developed for this purpose. The decoy strategy is applied to block TF activities by introduction of exogenous double-stranded oligodeoxynucleotide (ODN), which contains a consensus binding sequence of the target TF. In this way, ODN can compete with promoter region for binding to TF and work as so-called a decoy [2]. However, one of the defects of traditional decoy ODN is that it has limited ability of nuclear entry, which affects TF-ODN binding efficiency [3].

In light of the short hairpin RNA (shRNA) mediated RNA interference pathway, we noticed that shRNA is transcribed in nucleus, which means a temporary double-stranded RNA (dsRNA) molecule is formed before Exportin 5 mediated transportation to the cytoplasm, Dicer/TRBP/PACT complex mediated maturation and RISC complex mediated RNA interference [4]. We postulated that if this dsRNA contains consensus sequence of TF, except that *T* is replaced with *U*, it could also function as a decoy to block TF activities.

In the present study, we designed shRNA vectors containing consensus-binding sequences of several TFs and investigated whether they could block corresponding TFs activities as we hypothesized. This kind of shRNA vector was called vector based ODN (VB-ODN) here. Since Nuclear factor-κB (NF-κB) is a widely studied TF and its role in multiple cellular processes such as inflammatory response and carcinogenesis is relatively well understood, it is always chosen as a sample TF to test effects of newly-designed decoy [5,6]. Therefore, our study mainly evaluated effects of VB-ODN for NF-κB. We observed that in Human embryonic kidney 293 (HEK293) cells, VB-ODN carrying NF-κB consensus sequences decreased cell viability and downstream gene expression of NF-κB and the specific binding between dsRNA and NF-κB was confirmed by electrophoretic mobility shift assay (EMSA). Furthermore, the similar design also worked in other TFs indicating that this strategy could be applied as a common method to block TF activities.

Abbreviations: VB-ODN, vector-based oligodeoxynucleotide; TF, transcription factor; NF-κB, Nuclear factor-κB; TFD, transcription factor decoy; ODN, oligodeoxynucleotide; shRNA, short hairpin RNA; dsRNA, double-stranded RNA; HEK293 cells, human embryonic kidney 293 cells; EMSA, electrophoretic mobility shift assay; AP-2α, Activator Protein 2α; RXRA, retinoid X receptor alpha; NCBI, National Center for Biotechnology Information; IL-8, Interleukin-8; MCP-1, monocyte chemoattractant protein-1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; VEGF-D, Vascular endothelial growth factor D; IL-2, Interleukin-2; ODC1, ornithine decarboxylase 1; VDR, vitamin D (1,25-dihydroxyvitamin D3) receptor; SCD, stearoyl-CoA desaturase; NLS, nuclear location signal; miRNA, microRNA.

* Corresponding authors. Fax: +86 29 82539041.
E-mail addresses: lxuhelen@163.com (L. Xu), liuzhiguo@fmmu.edu.cn (Z. Liu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.12.091
0006-291X/

2. Materials and methods

2.1. Cell culture and transfection

HEK293 cells were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (CAS, Shanghai, China). Cells were cultured in DMEM supplemented with 10% fetal bovine serum and kept at 37 °C in an atmosphere with 5% CO_2 and 85% humidity. To introduce shRNA expression vector and ODNs into cells, Lipofectamine 2000 (Invitrogen, Carlsbad, CA, US) was used per the manufacture's protocols. Specifically, for 96-well plate, a transfection mix of 0.2 μg vector/0.5 μl Lipofectamine 2000 or 0.5 μg ODN/0.5 μl Lipofectamine 2000 was used for one well; for 6-well plate, a transfection mix of 4 μg vector/10 μl Lipofectamine 2000 or 10 μg ODN/10 μl Lipofectamine 2000 was used for one well.

2.2. Vector construction and ODN synthesis

For shRNA expression, a pTER$^+$ vector (www.addgene.org) was applied as the backbone. The following sequences corresponding to specific cis-element of NF-κB p50 were synthesized: 5′-GATCC CGGAATTTCCTTTCGAAGAGAGGAAATTCCTTTTTGGAAA-3′ and 5′-AGCTTTTCCAAAAAGGAATTTCCTCTCTTCGAAAGGAAATTCCGG-3′. The sequences were annealed and ligated between Bgl II and Hind III restriction sites. The resultant vector was used to produce decoy dsRNA. The following sequences were synthesized to construct its scrambled control vector: 5′-GATCCCTCGAGATCTTTTCGAAGAGAAGATCTCGATTTTTGGAAA-3′ and 5′-AGCTTTTCCAAAAATCGAGATCTTTCTCTTCGAAAAGATCTCGAGG-3′. A shRNA vector for NF-κB silencing served as a positive control. The sequences for this vector were: 5′-GATCCCGGGGCTATAATCCTGGACTTTCGAAGAGAGTCCAGGATTATAGCCCCTTTTTGGAAA-3′ and 5′-d(AGCTTTTCCAAAAAGGGGCTATAATCCTGGACTCTCTTCGAAAGTCCAGGATTATAGCCCCGG)-3′. And sequences for its relative scrambled control were: 5′-GATCC CTCGAGATCTTTTCGAAGAGAAGATCTCGATTTTTGGAAA-3′ and 5′-AGCTTTTCCAAAAATCGAGATCTTCTCTTCGAAAAGATCTCGAGG-3′ [7].

Sequences with the above mentioned cis-element of NF-κB were synthesized as a positive control ODN: 5′-GGAATTTCCTGGAATTTCCT-3′ and 5′-AGGAAATTCCAGGAAATTCC-3′, and the following sequences served as its scrambled control: 5′-TCGAGATCTTTCGAGATCTT-3′ and 5′-AAGATCTCGAAAGATCTCGA-3′. Two copies of the cis-element were involved in the positive control ODN according to previous study to enhance its decoy potency [8]. The consensus binding sequences, gene silencing sequences and their scrambled sequences were underlined. Above sequences for FOS, Activator Protein 2α (AP-2α) and retinoid X receptor alpha (RXRA) were listed in Supplementary Table 1. To eliminate potential interference effect of VB-ODN to other genes, sequences inserted to this vector were compared to the library of human genome by BLAST algorism provided by National Center for Biotechnology Information (NCBI) website to exclude unexpected binding. All sequences above were synthesized by Sangon Biotech Company (Shanghai, China).

2.3. RNA extraction and real-time RT-PCR

Cells were grown in 6-well plates and transfected with indicated vectors or ODNs. After 48 h, cells were collected and subjected to RNA extraction using E. Z. N. A.™ Total RNA Kit I (Omega Bio-tek, GA, US). Then 500 ng RNA was subjected to reverse transcription using TaKaRa PrimeScript RT reagent kit (TaKaRa Biotechnology, Dalian, China). PCR analysis was performed using SYBR Green Master Mix Kit (TaKaRa) for 15 min at 95 °C, followed by 40 cycles of 95 °C for 30 s and 60 °C for 30 s in an ABI Prism 7700 sequence Detection System. The fold-change of each mRNA was calculated using the $2^{-\triangle\triangle CT}$ method. The β-actin mRNA was set as the internal control and the final expression level of each gene was normalized to the control group. The primer sequences used for PCR analysis were listed in Supplementary Table 2.

2.4. Western blotting

Cells were lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) containing protease inhibitor cocktail tablet (Roche Applied Science, Mannheim Germany). Cell lysates were separated by 10% Sodium dodecyl sulfate–poly-acrylamide gel electrophoresis and were transferred to polyvinylidene difluoride membranes. Tris-buffered saline containing 5% non-fat milk powder and 0.1% Tween-20 was used for blocking. The membranes were then incubated with primary antibodies against NF-κB p50, Interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1) (Boster Biological Engineering Company, Wuhan, China) or β-actin (Sigma–Aldrich, St. Louis, MO, US) followed by incubation with secondary goat anti-rabbit IgG conjugated with horseradish peroxidase (Boster Biological Engineering Company). The protein blots were detected by enhanced chemiluminescence in BIO-RAD ChemiDoc XRS Imaging system.

2.5. MTT proliferation assay

Cells were seeded in 96-well plates at a density of 5000 cells per well. Transfection with vectors or ODNs was performed. Cell viability was measured 24 h, 48 h and 72 h after transfection. Briefly, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) solution (5 mg/ml) was added into wells (10 μl per 100 μl medium) followed by 4 h incubation at 37 °C. Then the solution was removed and dimethyl sulfoxide was added. The absorbance at 490 nm of each well was read on a microplate reader. Each group included 5 repeated wells and the experiment was performed in triplicate.

2.6. Apoptosis assay

Apoptosis assay was performed as described previously [9]. Briefly, after transfection of vectors or ODNs for 24 h, 50 μM etoposide was added to cell culture to induce apoptosis. 24 h later, cells were collected and stained with Annexin V-FITC and propidium iodide and apoptosis ratio was analyzed by flow cytometry.

2.7. Luciferase reporter assay

Luciferase reporter assay was conducted to measure the transcriptional activity of IL-8 and MCP-1. According to previous studies, NF-κB cis-elements at the promoter regions of IL-8 gene (−133 to −50 bp) and MCP-1 gene (−136 to +24 bp) were cloned into pGL3-basic vector [10]. Then pGL3-IL-8-Luc or pGL3- MCP-1-Luc was cotransfected into HEK293 cells with indicated vectors or ODNs mentioned above. pRL-TK Renilla was also transfected to serve as an internal control. A luciferase assay kit (Promega, Madison, Wis, US) and a Varioskan™ Flash Multimode Reader (Thermo Fisher Scientific, MA, US) were used to perform the luciferase reporter assay. The results were normalized with each control group set as 100%.

2.8. Electrophoretic mobility shift assay

Binding between dsRNA and NF-κB was detected using electrophoretic mobility shift assay (EMSA) as described previously [9]. The probe sequences were the same as ODN sequence mentioned

above with the dsRNA probe using *U* to replace *T*. Unlabeled dsRNA was added to the reaction mixture in a 100-fold excess to perform competition assays.

2.9. Statistical analysis

Data were expressed as mean ± SD from at least three independent experiments. Differences were compared by Student's *t*-test using SPSS 19.0 software (SPSS Inc., Chicago, IL, US). A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. NF-κB VB-ODN decreases survival of HEK293 cells

To investigate whether VB-ODN could exert a decoy effect, VB-ODN including NF-κB cis-element and its relative scrambled control vector were transfected into HEK293 cells, which were well known for the high transfection efficiency [11]. To provide positive controls, shRNA vector producing small interference RNA for NF-κB, ODN with double copies of NF-κB cis-elements and their relative scrambled control were also transfected into HEK293 cells. After transfection, it could be seen that shRNA significantly decreased NF-κB mRNA level (Fig. 1B) and also NF-κB p50 protein level (Fig. 1B) compared to its scrambled control while other transfection groups showed no noticeable changes in mRNA or protein level of NF-κB. Since NF-κB is a broadly investigated transcription factor, and its effects in proliferation inhibition and apoptosis induction were well established [12,13], proliferation and apoptosis of HEK293 cells were investigated to see if the NF-κB VB-ODN could work as a decoy. On a three-day proliferation assay, VB-ODN group demonstrated decreased proliferation ability compared to its scrambled control and this effect was similar to those achieved by NF-κB shRNA and ODN (Fig. 1C). In addition, the apoptotic ratio of cells was increased by VB-ODN, shRNA or ODN transfection (Fig. 1D).

3.2. VB-ODN decreases transcriptional activities of downstream genes of NF-κB

NF-κB could transcriptionally activate IL-8 and MCP-1 [14]. Therefore, to evaluate decoy activity of VB-ODN, we detected transcriptional activities of IL-8 and MCP-1.

The luciferase reporter assay showed that after VB-ODN, shRNA or ODN transfection, transcriptional activities of IL-8 and MCP-1were decreased to around 50–60% compared to their relative

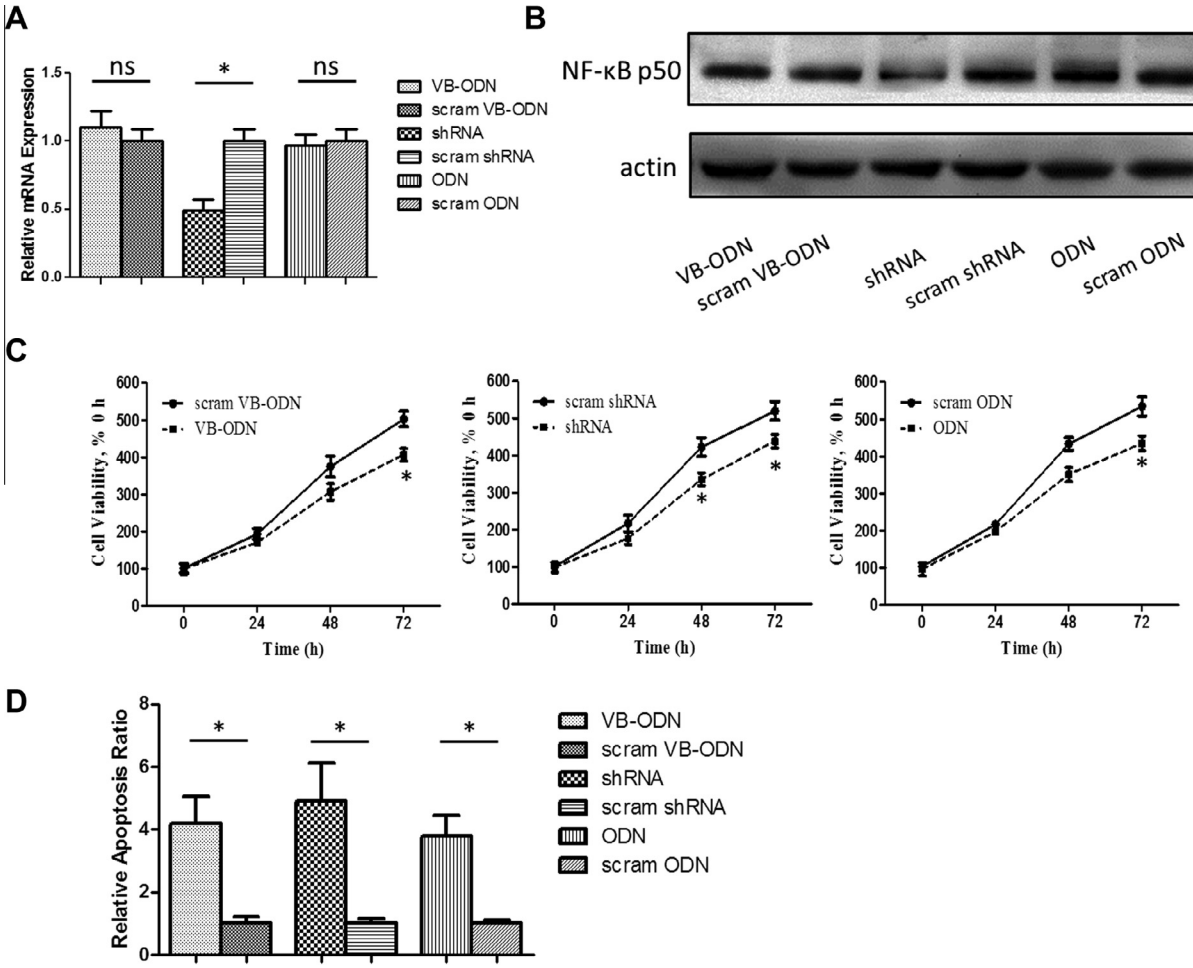

Fig. 1. NF-κB VB-ODN decreased HEK293 cell viability without affecting NF-κB expression. (A) Real-time RT-PCR revealed that VB-ODN and ODN did not affect NF-κB mRNA level compared to their scrambled control respectively, while shRNA group demonstrated a decreased level of NF-κB compared to its control. (B) Western blotting results further confirmed that the protein level of NF-κB was downregulated by shRNA but not by either VB-ODN or ODN. (C) Cell proliferation was inhibited after treatment with VB-ODN, shRNA or ODN during the three day observation reflected by MTT assay. The absorbance values at day 0 were set as 100%. (D) Cell apoptotic ratio was increased after transfection with VB-ODN, shRNA or ODN compared to their relative control respectively, with the apoptotic ratios of control groups set as 1. Differences between indicated groups were compared by *t*-test and all data were mean ± SD from three independent experiments. $^{*}P < 0.05$; ns: non-significant. VB-ODN: vector-based oligodeoxynucleotide, scram VB-ODN: scrambled control for VB-ODN, shRNA: short hairpin RNA, scram shRNA: scrambled control for shRNA, ODN: oligodeoxynucleotide, scram ODN: scrambled control for ODN.

control groups (Fig. 2A). To confirm this finding, we also detected mRNA level and protein level of IL-8 and MCP-1 and the results showed that blocking NF-κB activity by VB-ODN decreased both mRNA and protein levels of IL-8 and MCP-1, which was similar to the effects realized by shRNA knockdown or ODN blocking (Fig. 2B).

3.3. Verification of the ability of dsRNA to bind to NF-κB

We designed the VB-ODN and expected it to work as a TFD based on the hypothesis that dsRNA produced by it with the TF consensus binding sequence (except that *T* is replaced by *U*) may compete with the endogenous TF binding to block the TF activity. Therefore, evidence of direct binding between dsRNA and TF was needed to corroborate this notion. To this end, we performed EMSA assay and demonstrated that dsRNA carrying NF-κB consensus binding sequence was capable of binding to NF-κB, as indicated by the appearance of the shifted band, which was abrogated by a 100× unlabeled dsRNA competition but not by scrambled dsRNA. A labeled decoy ODN served as the positive control (Fig. 3). The results indicated that dsRNA could bind to NF-κB and work as a TFD as the ODN does.

3.4. VB-ODN decreases expression of downstream genes of FOS, AP-2α and RXRA

The above findings in NF-κB encouraged us to investigate if other TFs could also be blocked by the same VB-ODN design strategy. Thus, we randomly chose three other TFs expressed by HEK293 cells to see if the corresponding VB-ODNs could block their activities. After transfection, FOS mRNA expression was not affected by VB-ODN or ODN but significantly downregulated by shRNA (Fig. 4A). Vascular endothelial growth factor D (VEGF-D) and Interleukin-2 (IL-2), two downstream genes of FOS [15,16] showed decreased mRNA level after VB-ODN, shRNA or ODN transfection (Fig. 4B). We also detected mRNA levels of AP-2α, RXRA and their downstream genes, Cyclin B1 [17], ornithine decarboxylase 1 (ODC1) [18], vitamin D (1,25-dihydroxyvitamin D3) receptor (VDR) [19] and stearoyl-CoA desaturase (SCD) [20] after corresponding VB-ODN, shRNA and ODN transfection, and observed similar results with above findings (Fig. 4C–F).

4. Discussion

In the current study, we designed a new type of TFD utilizing the mechanism of shRNA expression. Instead of working as a tool for RNA interference, the shRNA bearing TF cis-elements herein could work as a TFD.

The ODN based TFD strategy was first described by Bielinska and coworkers [21]. Since TFD strategy was introduced, lots of novel approaches based on this design appeared. It has been reported that single-stranded DNA containing forward sequence of cis-elements has similar decoy function with traditional ODN and ODN bearing repeated sequences of cis-elements is more efficient in blocking target TFs [8]. In fact, TFD strategy has been used to suppress specific gene expression both *in vitro* and *in vivo* and it

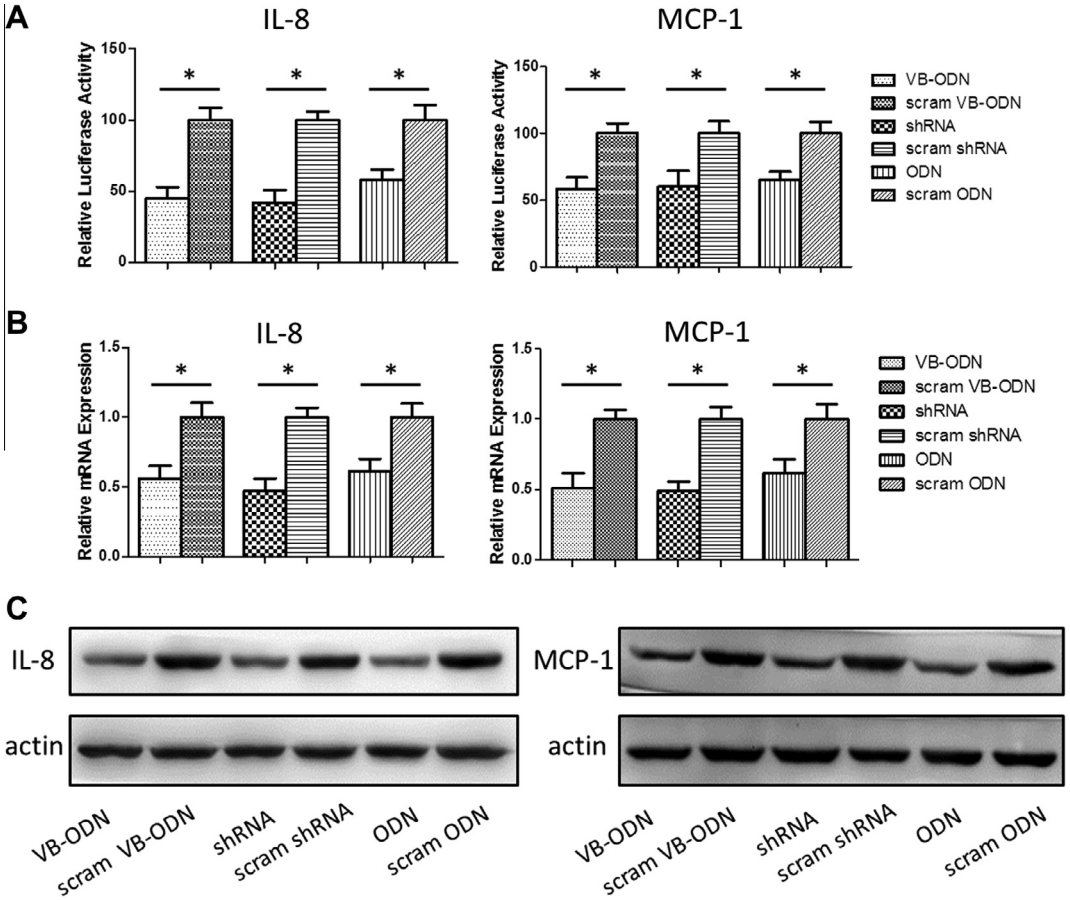

Fig. 2. NF-κB VB-ODN downregulated its downstream gene expression by transcriptional inhibition. (A) VB-ODN decreased transcriptional level of IL-8 and MCP-1 reflected by luciferase reporter assay as shRNA and ODN group did. The transcriptional activities of all control groups were set as 100. (B, C)VB-ODN decreased mRNA (B) and protein level (C) of IL-8 and MCP-1. The mRNA levels of all control groups were set as 1. Differences between indicated groups were compared by *t*-test and all data were mean ± SD from three independent experiments. $^{*}P < 0.05$; ns: non-significant. VB-ODN: vector-based oligodeoxynucleotide, scram VB-ODN: scrambled control for VB-ODN, shRNA: short hairpin RNA, scram shRNA: scrambled control for shRNA, ODN: oligodeoxynucleotide, scram ODN: scrambled control for ODN.

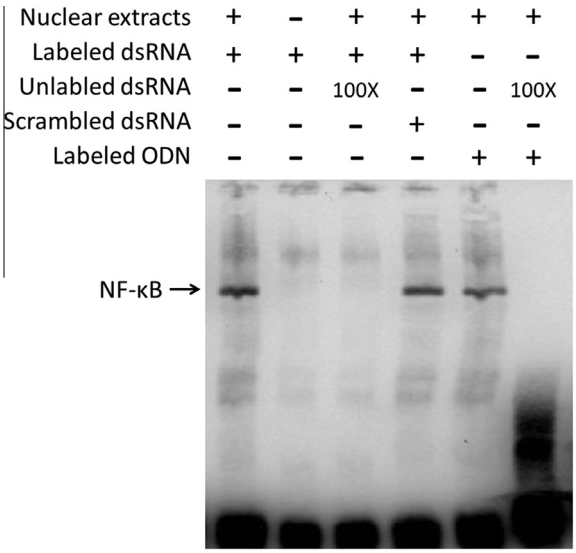

Fig. 3. Verification of the ability of dsRNA to bind to NF-κB. Labeled dsRNA caused the appearance of shifted band (Lane 1) which could neither be seen in negative control group (Lane 2) nor in unlabeled dsRNA competition group (Lane 3). Scrambled dsRNA did not affect the dsRNA-NF-κB binding (Lane 4). Labeled ODN served as the positive control (Lane 5) and the shifted band caused by it was also abrogated by dsRNA competition (Lane 6).

also demonstrated great potential as a novel therapeutic approach [22].

Although TFD strategy demonstrates promising advantages, some defects of traditional methods cannot be ignored. A conspicuous shortcoming of decoy ODN is the limited ability of nuclear entry. In fact, almost 90% of ODNs are assimilated and degraded by cytolysosome, which significantly restricts nuclear entry of ODN and further limits the amount of ODN-TF binding [22]. To overcome this defect, much effort was made to reduce cytolysosome degradation and enhance nuclear entry of ODN [23,24]. For example, nuclear location signal (NLS) was introduced, which could help ODN cross the nuclear membrane by anchoring on the membrane through interaction with NLS receptors on nuclear membrane. However, the ligation of ODNs and NLS peptides is not an easy reaction to perform [25,26].

The VB-ODN design in the present study took use of the characteristic of shRNA transcription and the similarity between dsRNA structure and ODN. As we expected, VB-ODN could work as a decoy.

One of the advantages of VB-ODN is that the backbone vector could be further packaged into virus particles and the virus mediated transduction of VB-ODN could dramatically increase the efficacy of shRNA expression and enhance the TF blocking efficiency. And if lenti-virus system is deployed, the transient TFD effect could be transformed to a long-term manipulation. As a result, the barrier of cytolysosome degradation could be overcome in this way. But this deduction, however reasonable, still needs corroboration by further tests.

In a recent report, Cui and co-workers demonstrated that micr-oRNA (miRNA) could work as endogenous decoy to regulate gene transcriptional expression. This finding emphasized that besides participating in post-transcriptional regulation, miRNA could also act as a transcriptional regulator if it contains cis-elements sequence of some TFs. This kind of miRNA sharing similar structure

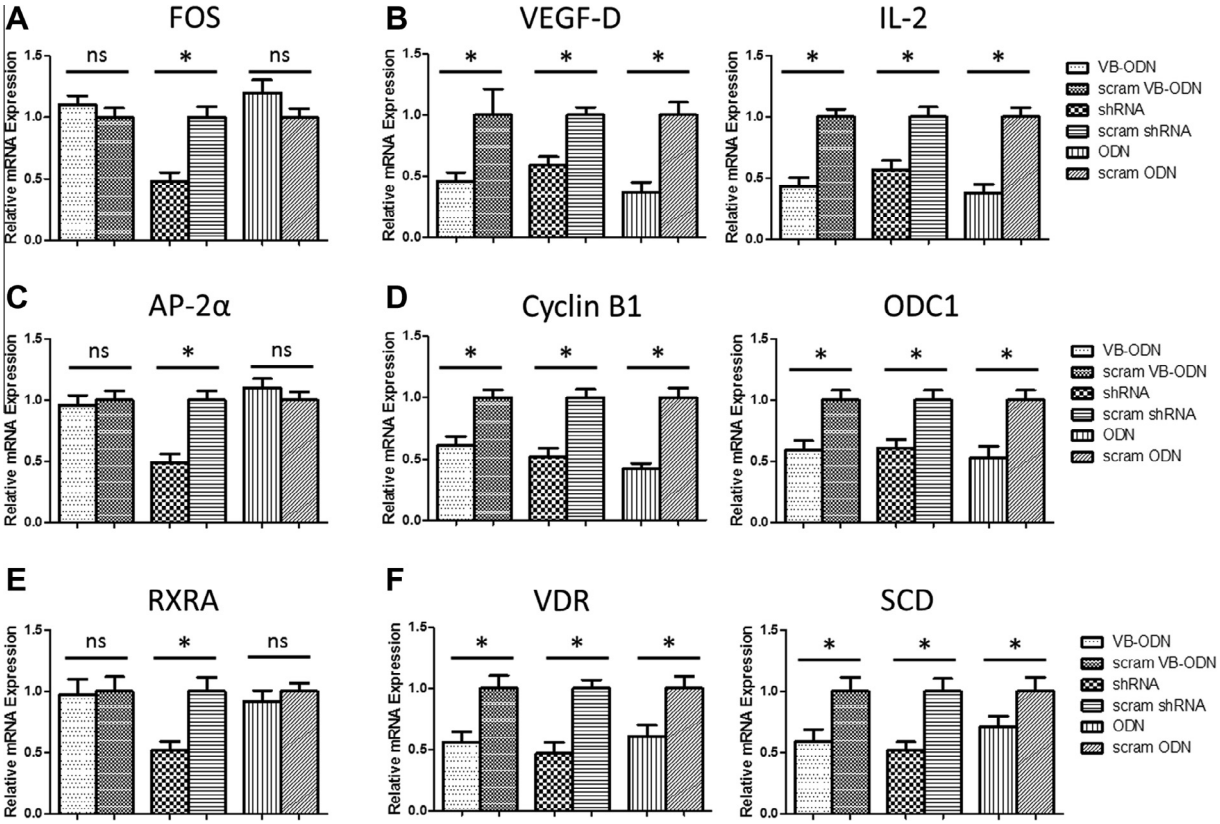

Fig. 4. VB-ODN designed for FOS, AP-2α and RXRA decreased their downstream gene expression but not that of themselves. (A) FOS mRNA level was decreased by shRNA but not by VB-ODN or ODN. (B) VEGF-D and IL-2 mRNA levels were decreased by VB-ODN, shRNA and ODN. (C) AP-2α mRNA level was decreased by shRNA but not by VB-ODN or ODN. (D) Cyclin B1 and ODC1 mRNA levels were decreased by VB-ODN, shRNA and ODN. (E) RXRA mRNA level was decreased by shRNA but not by VB-ODN or ODN. (F) VDR and SCD mRNA levels were decreased by VB-ODN, shRNA and ODN. Differences between indicated groups were compared by *t*-test and all data were mean ± SD from three independent experiments. $^{*}P < 0.05$; ns: non-significant. VB-ODN: vector-based oligodeoxynucleotide, scram VB-ODN: scrambled control for VB-ODN, shRNA: short hairpin RNA, scram shRNA: scrambled control for shRNA, ODN: oligodeoxynucleotide, scram ODN: scrambled control for ODN.

with ODNs could work as a decoy to compete for the TF binding and regulate downstream gene expression [27]. Our design in this study was based on the mechanism described above, but we focused on adopting it as an exogenous tool to manipulate genes regulated by TFs.

In fact, RNA–protein interaction happens in many cellular processes such as alternative splicing, RNA editing, protein translation, etc [28,29]. In our study, we proposed to use this interaction to modify traditional TFD strategy. EMSA results demonstrated that dsRNA transcribed from VB-ODN did specifically bind to target TF and this binding will occupy the DNA binding domain in TF leading to attenuated TF–ODN interaction. We also used NCBI blast function to exclude the possibility of non-specific binding to ensure that the cellular phenotype changes or downstream gene suppression related to the target TF was a relevant effect caused by TF activity attenuation other than potential RNA interference.

In addition to investigating the effect of VB-ODN for NF-κB, we also inspected effects of VB-ODNs for three other randomly chosen TFs in HEK293 cells. Although we did not detect related cellular phenotypes and dsRNA-TF binding for all of them, we did find downstream genes changes in agreement with TF activity inhibition. This implicated that the VB-ODN could be further investigated in other TFs and be developed into a universal tool for gene regulation.

Another limitation of our study is that we did not quantify the transfection efficiency especially the nuclear abundance of VB-ODN, shRNA or ODN. Although the cell line we used, HEK293, allowed a high efficiency of exogenous nucleotide transfection thus providing a relatively reliable comparison results between VB-ODN and its negative or positive controls, more precise indicators such as fluorescence or biotin label could be introduced to the vectors or ODNs to reflect the transfection efficiency, especially for the future exploration in other cell lines to study specific biological or pathological behaviors.

In conclusion, we propose a new design of TFD, VB-ODN, to block TF activities. This new approach utilizes mechanism of shRNA vector transfection and the following shRNA transcription in nucleus to achieve a dsRNA based TFD strategy (Fig. S1). This strategy provides possibility of viral particle production, resulting in enhanced TFD nuclear entry efficiency and therefore will be applicable to TFD-based clinical practices.

Conflict of interest

None.

Acknowledgment

This work was supported by National Natural Sciences Foundation of China, Nos. 81272449, 81372387, 81472300 and 81400632.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.12.091.

References

[1] G. Cabrini, V. Bezzerri, I. Mancini, et al., Targeting transcription factor activity as a strategy to inhibit pro-inflammatory genes involved in cystic fibrosis: decoy oligonucleotides and low-molecular weight compounds, Curr. Med. Chem. 17 (2010) 4392–4404.
[2] M.J. Mann, Transcription factor decoys: a new model for disease intervention, Ann. N. Y. Acad. Sci. 1058 (2005) 128–139.
[3] M.J. Cairns, E.G. Saravolac, L.Q. Sun, Catalytic DNA: a novel tool for gene suppression, Curr. Drug Targets 3 (2002) 269–279.
[4] D.D. Rao, J.S. Vorhies, N. Senzer, et al., SiRNA vs. shRNA: similarities and differences, Adv. Drug Deliv. Rev. 61 (2009) 746–759.
[5] Y. Higuchi, K. Furukawa, T. Miyazawa, et al., Development of a new dumbbell-shaped decoy DNA using a combination of the unnatural base pair ImO(N):NaN(O) and a CuAAC reaction, Bioconjug. Chem. 25 (2014) 1360–1369.
[6] Y. Kono, S. Kawakami, Y. Higuchi, et al., Antitumor effect of nuclear factor-kappaB decoy transfer by mannose-modified bubble lipoplex into macrophages in mouse malignant ascites, Cancer Sci. 105 (2014) 1049–1055.
[7] A. Arlt, M.L. Kruse, M. Breitenbroich, et al., The early response gene IEX-1 attenuates NF-kappaB activation in 293 cells, a possible counter-regulatory process leading to enhanced cell death, Oncogene 22 (2003) 3343–3351.
[8] J.J. Cooper, J.M. Parks, R. Carcelen, et al., Attenuation of interleukin-8 production by inhibiting nuclear factor-kappaB translocation using decoy oligonucleotides, Biochem. Pharmacol. 59 (2000) 605–613.
[9] T. Li, H. Wang, Y. Sun, et al., Transcription factor CUTL1 is a negative regulator of drug resistance in gastric cancer, J. Biol. Chem. 288 (2013) 4135–4147.
[10] J.X. Bai, B. Yan, Z.N. Zhao, et al., Tamoxifen represses miR-200 microRNAs and promotes epithelial-to-mesenchymal transition by up-regulating c-Myc in endometrial carcinoma cell lines, Endocrinology 154 (2013) 635–645.
[11] P. Thomas, T.G. Smart, HEK293 cell line: a vehicle for the expression of recombinant proteins, J. Pharmacol. Toxicol. Methods 51 (2005) 187–200.
[12] L. Penolazzi, E. Lambertini, M. Borgatti, et al., Decoy oligodeoxynucleotides targeting NF-kappaB transcription factors: induction of apoptosis in human primary osteoclasts, Biochem. Pharmacol. 66 (2003) 1189–1198.
[13] M. Shibata, T. Sato, R. Nukiwa, et al., TRIM45 negatively regulates NF-kappaB-mediated transcription and suppresses cell proliferation, Biochem. Biophys. Res. Commun. 423 (2012) 104–109.
[14] D.R. Clifton, E. Rydkina, H. Huyck, et al., Expression and secretion of chemotactic cytokines IL-8 and MCP-1 by human endothelial cells after *Rickettsia rickettsii* infection: regulation by nuclear transcription factor NF-kappaB, Int. J. Med. Microbiol. 295 (2005) 267–278.
[15] M. Orlandini, L. Marconcini, R. Ferruzzi, et al., Identification of a c-Fos-induced gene that is related to the platelet-derived growth factor/vascular endothelial growth factor family, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 11675–11680.
[16] A. Mondino, C.D. Whaley, D.R. DeSilva, et al., Defective transcription of the IL-2 gene is associated with impaired expression of c-Fos, FosB, and JunB in anergic T helper 1 cells, J. Immunol. 157 (1996) 2048–2057.
[17] J.P. Cogswell, M.M. Godlevski, M. Bonham, et al., Upstream stimulatory factor regulates expression of the cell cycle-dependent cyclin B1 gene promoter, Mol. Cell. Biol. 15 (1995) 2782–2790.
[18] A.E. Pegg, Regulation of ornithine decarboxylase, J. Biol. Chem. 281 (2006) 14529–14532.
[19] J. Zhang, M.J. Chalmers, K.R. Stayrook, et al., DNA binding alters coactivator interaction surfaces of the intact VDR–RXR complex, Nat. Struct. Mol. Biol. 18 (2011) 556–563.
[20] E. Schmitt, M.A. Ballou, M.N. Correa, et al., Dietary lipid during the transition period to manipulate subcutaneous adipose tissue peroxisome proliferator-activated receptor-gamma co-regulator and target gene expression, J. Dairy Sci. 94 (2011) 5913–5925.
[21] A. Bielinska, R.A. Shivdasani, L.Q. Zhang, et al., Regulation of gene expression with double-stranded phosphorothioate oligonucleotides, Science 250 (1990) 997–1000.
[22] M.J. Mann, V.J. Dzau, Therapeutic applications of transcription factor decoy oligonucleotides, J. Clin. Invest. 106 (2000) 1071–1075.
[23] E. Fattal, G. Barratt, Nanotechnologies and controlled release systems for the delivery of antisense oligonucleotides and small interfering RNA, Br. J. Pharmacol. 157 (2009) 179–194.
[24] V.J. Dzau, M.J. Mann, R. Morishita, et al., Fusigenic viral liposome for gene therapy in cardiovascular diseases, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 11421–11425.
[25] M.A. Zanta, P. Belguise-Valladier, J.P. Behr, Gene delivery: a single nuclear localization signal peptide is sufficient to carry DNA to the cell nucleus, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 91–96.
[26] C. Boulanger, C. Di Giorgio, P. Vierling, Synthesis of acridine-nuclear localization signal (NLS) conjugates and evaluation of their impact on lipoplex and polyplex-based transfection, Eur. J. Med. Chem. 40 (2005) 1295–1306.
[27] C. Cui, J. Yu, S. Huang, et al., Transcriptional regulation of gene expression by microRNAs as endogenous decoys of transcription factors, Cell. Physiol. Biochem. 33 (2014) 1698–1714.
[28] T. Glisovic, J.L. Bachorik, J. Yong, et al., RNA-binding proteins and post-transcriptional gene regulation, FEBS Lett. 582 (2008) 1977–1986.
[29] G. Dreyfuss, V.N. Kim, N. Kataoka, Messenger-RNA-binding proteins and the messages they carry, Nat. Rev. Mol. Cell Biol. 3 (2002) 195–205.