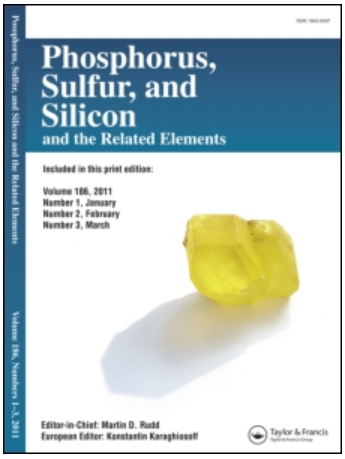

Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Antisense Therapy Targeting Pokemon Oncogene in MCF-7 Cells

Nan Zhang[ab]; Zhenhua Xie[b]; Yuyang Jiang[abc]; Yufen Zhao[ad]
[a] Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education, Department of Chemistry Tsinghua University, Beijing, P. R. China [b] Key Laboratory for Chemical Biology of Guangdong Province, Shenzhen Graduate School of Tsinghua University, Shenzhen, P. R. China [c] School of Medicine, Tsinghua University, Beijing, China [d] Key Laboratory for Chemical Biology of Fujian Province, Department of Chemistry Xiamen University, Xiamen, P. R. China

To cite this Article Zhang, Nan , Xie, Zhenhua , Jiang, Yuyang and Zhao, Yufen(2008) 'Antisense Therapy Targeting Pokemon Oncogene in MCF-7 Cells', Phosphorus, Sulfur, and Silicon and the Related Elements, 183: 2, 755 — 760
To link to this Article: DOI: 10.1080/10426500701807822
URL: http://dx.doi.org/10.1080/10426500701807822

Phosphorus, Sulfur, and Silicon, 183:755–760, 2008

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500701807822

Taylor & Francis
Taylor & Francis Group

Antisense Therapy Targeting Pokemon Oncogene in MCF-7 Cells

Nan Zhang,[1,2] Zhenhua Xie,[2] Yuyang Jiang,[1,2,4] and Yufen Zhao[1,3]

[1]Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Department of Chemistry Tsinghua University, Beijing, P. R. China
[2]Key Laboratory for Chemical Biology of Guangdong Province, Shenzhen Graduate School of Tsinghua University, Shenzhen, P. R. China
[3]Key Laboratory for Chemical Biology of Fujian Province, Department of Chemistry Xiamen University, Xiamen, P. R. China
[4]School of Medicine, Tsinghua University, Beijing, China

This study is undertaken to investigate the role of Pokemon oncogene in MCF-7 cells growth and the potential of Pokemon as a target for cancer therapy. An 18-mer antisense oligonucleotide As4 (TTCAGGTCGTAGTTGTGG, targeting 1496–1513 site of Pokemon mRNA) has been proven as a potent inhibitor of Pokemon expression in our previous work. In this study, we transfected As4 to MCF-7 cells and confirmed the Pokemon mRNA and protein level by RT–PCR and western blot. Furthermore, the cell cycle has been analyzed by flow cytometer. G_2/M arrest has been observed when the cells were treated with antisense oligonucleotide at 100 nM for 48 h. We've also found the P53 protein upregulation due to Pokemon inhibition. These results suggest that Pokemon inhibitor may be a novel approach to human cancer therapy.

Keywords Antisense oligonucleotides; cancer therapy; cell cycle; Pokemon; p53

INTRODUCTION

Antisense oligonucleotides can specifically hybridize and inhibit the expression of pathogenic genes, which is widely used in the gene therapy.[1—4] Recently, we have developed a series of 18-mer phosphorothioate oligonucleotides targeting coding regions of Pokemon mRNA.

The authors would like to thank the financial supports from the Chinese National Natural Science Foundation (20572060, 20472043), the Project of Science and Technology of Guangdong Province (2005A11601008), and Mega-Project of Science Research of Ministry of Science and Technology of China (2005CCA03400).

Address correspondence to Yuyang Jiang, Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Department of Chemistry Tsinghua University, Beijing 100084, P. R. China. E-mail: jiangyy@sz.tsinghua.edu.cn

Pokemon is a new oncogene, a gene that can mutate normal cells into cancerous cells, but its role is unique in that it controls the activity of other oncogenes. Pokemon is therefore a main switch in the molecular network that leads to cancer.

Pokemon is one member of the POK (POZ and Krüppel) protein family, which contains an NH_2-terminal POZ domain and a COOH-terminal DNA-binding domain. POK proteins are critical in embryonic development,[5,6] differentiation,[7,8] and oncogenesis.[9–11]

Recently, Pokemon has been found as a central regulator of the important tumor suppressor alternative reading frame (ARF). By binding the $p19^{Arf}$ promoter *in vivo*, Pokemon can repress ARF's activity.[11,12] It mediates cells' growth by the Pokemon-ARF-MDM2-P53 pathway. In view of its essential role in oncogenic transformation, Pokemon is an attractive therapeutic target for human cancer. In the present work, an 18-mer antisense oligonucleotide has been designed to inhibit Pokemon expression in order to test whether the latter correlates with cell cycle, senescence, and apoptosis.

MATERIALS AND METHODS

Antisense Oligonucleotides

As4, an 18-mer anti-human-Pokemon phosphorothioate oligonucleotide (*TTCA*GGTCGTAGTT*GTGG*, in italic is phosphorothioate linkage, targeting 1496–1513 sites of Pokemon mRNA) was designed and synthesized in our previous work. We've also synthesized an 18-mer mismatched oligonucleotide (*ATGT*AGTCGTCTGC*CTAG*, in italic is phosphorothioate linkage) as control.

Cell Culture and Tansfection

MCF-7 cells (American Type Culture Collection) were grown at 37°C in a 5% CO_2 atmosphere with in DMEM (Dulbecco's modied Eagle's medium), supplemented with 10% (V/V) fetal bovine serum (FBS), 100 mg/ml penicillin, and 100 mg/ml streptomycin. At 24 h before transfection, cells were seeded in 24-wells plates at a density of 1×10^5 cells/well in a volume of 500 μL.

For each transfection, antisense oligonucleotides were mixed with 50 μL of Opti-MEM (Invitrogen, USA) medium without serum. In a separate tube, 2.5 μL of Lipofectamine-2000^{TM} (Invitrogen, USA) was added to 50 ml of Opti-MEM medium and incubated for 5 min at room temperature. Two solutions were mixed gently, and incubated for an additional 20 min at room temperature. The mixed solution was then added to the cells in the 24-wells plates. At 6 h post-transfection, the

medium was replaced by normal medium containing 10% FBS and antibiotics and cultured for 24 h–60 h.

Western Blot Analysis

The protein level of Pokemon and P53 in cells treated with antisense oligonucleotides were analyzed by western blot. Cells were transfected with oligonucleotides as described above. After treated for 48 h, cells were lysed directly in 6-well plates with lysing solution (10 mmol/L, 150 mM NaCl, 0.5% NP-40, 1 mM), and the lysate was boiled for 10 min at 100°C. Equal amounts of protein were electrophoresed on 10% polyacrylamide gels. The separated proteins were transferred to nitrocellulose membranes (Pall Corporation, USA). The Pokemon expression was detected using the anti-Pokemon F9304 antibody (Sigma, USA) at a dilution of 1:4000, and the P53 expression was detected using the anti-P53 FL-393 antibody (Santa Cruz Biotechnology, USA) at a dilution of 1:500. Signals were collected by chemiluminesent (Pierce Biotechnology, Inc, USA).

Quantitation of mRNA

The mRNA level of Pokemon in cells treated with antisense oligonucleotides was analyzed by RT-PCR. Cells were transfected with oligonucleotides as described above. Total RNA was prepared by using the Trizol reagent (Invitrogen, USA), treated by DNasel, quantified by ultraviolet spectrophotometry, and was then used to create cDNA by using the ImProm-II™ Reverse Transcription System (Promega, USA). For quantitative analysis of Pokemon mRNA, human GAPDH mRNA was used as an internal control. The amplification of Pokemon cDNA was performed using 5′-TGCAAGGTCCGCTTCACCAG-3′ as a forward primer (fp), and 5′-GGCTGTGAAGTTACCGTCGGTG-3′ as a reverse primer (rp). The primers for GAPDH were 5′-CAACGTGTC AGTGGTGGACCTG-3′ (fp), and 5′-TTACTCCTTGGAGGCCATGTGG-3′ (rp), respectively. PCR was performed for Pokemon with cycling parameters of 98°C for 10 s, 40 cycles at 98°C for 10 s, 60°C for 10 s, 72°C for 40 s, and finally 72°C for 2 min. The cycling for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was essentially the same as for Pokemon except that the annealing was performed at 24 cycles. PCR products were run on a 1% agarose gel and visualized by ethidium bromide staining. Gel images were obtained and quantified using Quantity One analysis software (Bio-Rad, USA).

Flow Cytometry Snalysis

Cells were seeded in 24-well plates and transfected with antisense oligonucleotides as described above. After incubation for 48 h, cells

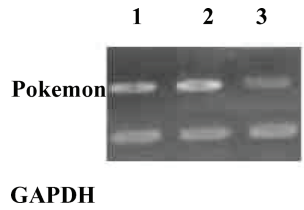

FIGURE 1 RT–PCR For Pokemon mRNA expression. Line 1, Lipofectamine 2000 only control; Line 2, mismatch control; Line 3, As4 treatment.

were harvested and resuspended in 1 ml of hypotonic propidium iodide (Sigma) solution (50 μg/ml) prepared in 0.1% sodium citrate plus 0.1% Triton X-100 and 100 mg/ml DNase-free RNase A for DNA staining. Stained cells were analyzed by flow cytometry (Becton Dickinson FACScan) at excitation 488/emmision 600 nm. Meanwhile, dead or abnormal cells were omitted by gating of side versus forward scatter and histograms of fluorescence intensity versus cell number were generated.

RESULTS AND DISCUSSION

Antisense Inhibition in MCF-7 Cells

In order to find out whether antisense oligonucleotide As4 can specifically reduce expression of Pokemon, RT-PCR and Western Blot were used to analyze the mRNA and protein levels of Pokemon, respectively. After incubation with As4 at 100 nM for 48 h, Pokemon mRNA level was reduced to 65%, whereas no reduced mRNA level was observed for cells treated with the mismatch control (Figure 1). Therefore, it is concluded that the observed antisense effects were the sequence specific degradation of Pokemon mRNA. The Pokemon protein level was also inhibited shown by western blot (Figure 2).

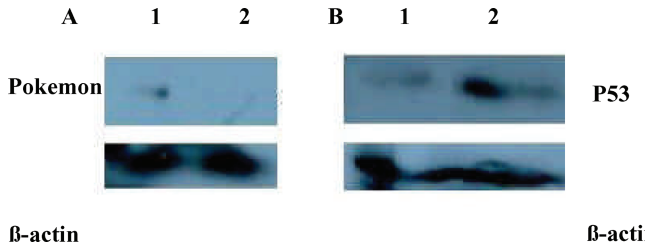

FIGURE 2 Western Blot for Pokemon protein expression. (A) Line 1, mismatch control; Line 2, As4 treatment. (B) Line 1, mismatch control; Line 2, As4 treatment.

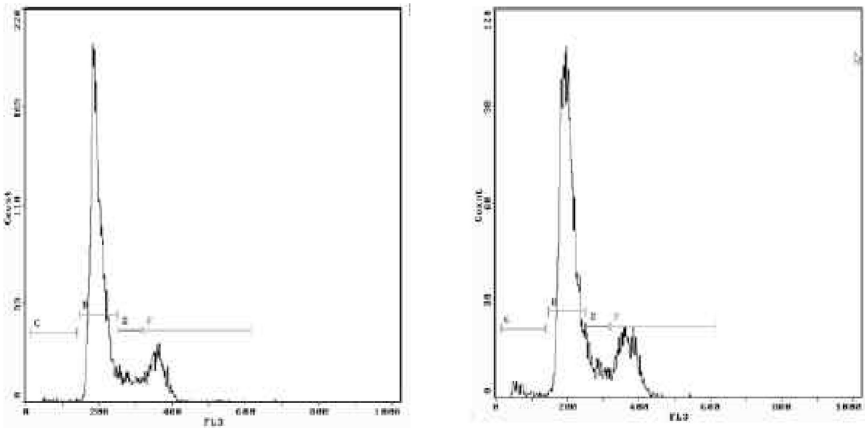

FIGURE 3 Flow cytometer analysis of cell cycle. (A) MCF-7 cells were treated with mismatch antisense oligonucleotide as control. (B) MCF-7 cells were treated with antisense oligonucleotide As4 at 100 nM for 48 h.

Furthermore, the result shows that activation of P53 was correlated with the Pokemon inhibition (Figure 2), in accordance with the Pokmon-ARF-MDM2-P53 pathway proposed by Maeda.[11]

Antisense Effect on Cell Cycle in MCF-7 Cells

In order to investigate the antisense effects on cell cycle, MCF-7 cells were transfected with antisense oligonucleotides at 100 nM. After the analysis of cell cycle distribution by flow cytometer, we've also found that the percentage of G_2/M phase increased along with the increase concentration of antisense oligonucleotide. The highest level of G_2/M arrest is observed when the cells are treated with antisense oligonucleotide at 100 nM for 48 h. Our result suggests that Pokemon might play a positive role in cell cycle, especially in the G_2/M phase (Figure 3).

CONCLUSION

In the present study, we've found antisense oligonucleotides As4 specifically knocked-down Pokemon expression at both mRNA and protein levels. Inhibition of Pokemon results in P53 protein upregulation and G_2/M arrest. These results provide a direct evidence for the function of Pokemon and it may be a novel approach to therapy of human cancer.

REFERENCES

[1] A. Dove, *Nat. Biotechnol.*, **20,** 121 (2002).
[2] H. Wang, G. Gautam, J. K. Buolamwini, et al., *Curr. Cancer Drug Tar.*, **1,** 177 (2001).
[3] S. Agrawal and E. R. Kandimalla, *Curr. Cancer Drug Tar.,* **1,** 197 (2001).
[4] J. B. Opalinska and A. M.Gewirtz, *Nat. Rev. Drug Discov.*, **1,** 503 (2002).
[5] M. Barna, N. Hawe, L. Niswander, et al., *Nat. Genet.*, **25,** 166 (2000).
[6] X. He, X. He,V. P. Dave, et al., *Nature*, **433,** 826 (2005).
[7] J. A. Costoya, R. H. Hobbs, M. Barna, et al., *Nat. Genet.*, **36,** 653 (2004).
[8] B. H. Ye, G. Cattoretti, Q. Shen, J. Zhang, et al., *Nat. Genet.*, **16,** 161 (1997).
[9] M. H. M. Koken, A. Reid, F. Quignon, et al., *Proc. Natl. Acad. Sci. U S A*, **94,** 10255 (1994).
[10] W. Y. Chen, X. Zeng, M. G. Carter, et al., *Nat. Genet.*, **33,** 197 (2003).
[11] T. Maeda, R. M. Hobbs, T. Merghoub, et al., *Nature*, **433,** 278 (2005).
[12] T. Maeda, R. M. Hobbs, and P. P. Pandolfi, *Cancer Res.*, **65,** 8575 (2005).