Int J Clin Exp Pathol 2018;11(6):3157-3164
www.ijcep.com /ISSN:1936-2625/IJCEP0076641

Original Article

The reactivation of P53 by saRNA affects the biological behavior in vitro in gastric cancer cells

Jing Wang[1*], Han Li[2*], Detian Xie[3], Leping Li[4], Jinshen Wang[4], Lipan Peng[4], Yanbing Zhou[2]

[1]Department of Human Anatomy, Qingdao University Medical College, Shandong, China; [2]Department of General Surgery, Affiliated Hospital of Qingdao University, Shandong, China; [3]Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Shandong, China; [4]Department of General Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Shandong, China. [*]Equal contributors and co-first authors.

Received February 1, 2018; Accepted April 11, 2018; Epub June 1, 2018; Published June 15, 2018

Abstract: This study sought to verify the reactivation effect of dsP53-285 that can up-regulate P53 expression in vitro. In addition, we explored the reactivation effect that dsP53-285 has on the biological behavior of gastric cancer cells. The specific small activating RNA (saRNA), dsP53-285, targeting the P53 gene promoter was synthesized. Also, a double strained control RNA (dsControl) was synthesized as a negative control, and then siP53 was synthesized to exclude the off-target effect. Both BGC-823 and MGC-803 cells were transfected with the corresponding microRNA, or just treated with lipofectamine2000. RT-qPCR and Western blot were adopted to detect P53 mRNA or the protein content of each group. CCK-8 was adopted to detect the proliferation of each group. The migration ability was assessed using the scratch-wound assay. The results of RT-qPCR and Western blot showed that dsP53-285 caused a significant up-regulation of the P53 gene ($P<0.01$), and the expression level of the P21 gene changed with the reactivation. The CCK-8 showed that, compared to the control group, the proliferation ability of the dsP53-285 group was inhibited significantly ($P<0.01$). The reactivation effect was in a time-course manner. The wound scratch assay proved that, compared to the control group, the migration ability of dsP53-285 group was inhibited significantly ($P<0.01$). This phenomenon provides a theoretical basis for the carcinostatic activity of small activating RNA (saRNA) and might indicate a new targeted treatment option for gastric cancer.

Keywords: Gastric cancer, saRNA, P53, biologic behavior

Introduction

Gastric cancer (GC) is one of the most common malignant tumors and causes of death worldwide, with about 951,000 cases diagnosed and 693,000 deaths in 2012 [1]. GC is a heterogeneous disease, and it is often diagnosed at an advanced stage, making it more difficult to cure [2]. Until now surgery has been the only method to cure GC, but just 40% of the patients have been relieved from GC after a complete resection of their tumor [2, 3]; the others who are diagnosed at an advanced stage have a median survival of less one year in spite of the targeted treatment options, including c-Met, epidermal growth factor receptor, immune checkpoint inhibition, poly ADP-ribose polymerase, and the phosphatadylinositol 3-kinase/protein kinase B/mammalian target of rapamycin pathway [4]. Therefore, there is a need for a new way to better define patient populations through targeted treatment options. Therefore, small activating RNAs (saRNAs), which can achieve the goal partly, have come to our attention. RNA activation (RNAa) can achieve an anticancer effect by reactivating target genes, usually the inactivated oncogenes.

Small activating RNAs (saRNAs) are 19- to 21-base-pair duplexes with a structure identical to siRNA, but saRNA can trigger gene activation or enhance gene expression at the transcription and post-transcription levels; the phenomenon is called RNA activation (RNAa) [5-7]. Currently, the design rule of saRNA and the mechanism of RNAa are still being explored. Different studies have confirmed that saRNA can trigger or enhance transcriptional activation by various pathways, mainly includ-

ing targeting specific sequences of promoters [8-12] and/or specific gene antisense transcripts [13-15]. Moreover, saRNA can enhance post-transcription expression in different ways [7].

The P53 gene is also called TP53. It is located on chromosome 17p13.1, and its role is to maintain the genomic stability by encoding the transcriptional factor, p53, which can regulate the cellular cycle and cause cellular apoptosis [16]. As a tumor suppressor gene, the wide-type is often mutated or inactivated in cancer, and the expression level of p53 has been implicated as an important predictor for patient progression and survival [17]. In addition, p21 is regulated by p53, the signal pathway that is related to the biological behavior of cancer [18-20].

However, the application of dsP53-285 has not been reported in gastric cancer. In this study, we transfected gastric cancer cells with dsP53-285, then explored the reactivation effect of saRNA in gastric cancer. This may provide a new cancer therapy.

Materials and methods

Reagents

The sequence of specific saRNA, dsP53-285, is as follows: S, 5'-UUACGGAAAGCCUUCCUAA[dT][dT]-3', AS, 5'-UUAGGAAGGCUUUCCGUAA[dT][dT]-3'; dsControl, S, 5'-ACUACUGAGUGACAGUAGA[dT][dT]-3', AS, 5'-UCUACUGUCACUCAGUAGU[dT][dT]-3'; siP53, S, 5'-CUACUUCCUGAAAACAACG[dT][dT]-3', AS, 5'-CGUUGUUUUCAGGAAGUAG[dT][dT]-3'. All of the sequences were synthesized by GenePharma (Shanghai, China).

Cell culture and transfection

The GC cell lines MGC-803 and BGC-823 were bought from ZIGB-BIO (Beijing, China). Both were cultured in RPMI-1640 (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, USA), and incubated at 37°C in a humidified atmosphere containing 5% CO_2. After two passages, the cells were transfected. The day before transfection, both kinds of cell lines were trypsinized, and seeded into a 6-well plate with Opti-MEM (Gibco, USA) without antibiotics, at an appropriate density. Both GC cell lines were cultured overnight until they reached 30%-40% confluence, and then transfected with dsP53-285, or dsControl at a concentration of 50 nM for 72 h by using Lipofectamine2000 (Invitrogen, USA) as a transfectant. The mock groups were just treated with Lipofectamine2000. After 72 h, we separated the dsP53-285 group into two groups; one was used for the experimental group, and another was transfected with siP53, which was used to eliminate the off-target effect.

Quantitative real-time PCR

Total RNA was extracted from each group by using TRIzol solution (TaKaRa RNAiso Plus). 1 μg of the RNA was used for cDNA synthesis using the TaKaRa PrimeScript RT reagent Kit with gDNA Eraser. The obtained cDNA was amplified using SYBR Premix Ex Taq (TaKaRa), combined with corresponding primer sets. Real-time PCR was performed in a 20 ml reaction system. The primers were as follows: P-53, forward, 5'-CAGCACATGACGGA GGTTGT-3', reverse, 5'-TCATCCAAATACTCCACACGC-3'; and GAPDH: forward, 5'-GGACCTGACCTGCCGTCTAG-3', and reverse, 5'-GTAGCCCAGGATGCCCTTGA-3'. The procedure followed the manufacturer's instructions.

Protein analysis by western blotting

Briefly, all the transfected cells were harvested at 72 h following dsRNAs treatment as described above, washed three times, and lysed with a lysis buffer. The protein concentration was determined using the bicinchoninic acid protein assay kit (Pierce Biotechnology, Rockford, IL). Equivalent amounts of protein were separated by electrophoresis on 10% SDS-polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membranes by voltage gradient-transfer. Membranes were blocked in 5% nonfat dry milk for 2 h at room temperature and then washed with TBST three times. The membranes were then incubated overnight with the appropriate primary antibody at specified dilutions. Then primary antibodies were removed, and the blots were extensively washed with TBST three times. Blots were then incubated for an hour at room temperature with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibody. After washing three times, the blots were detected using an

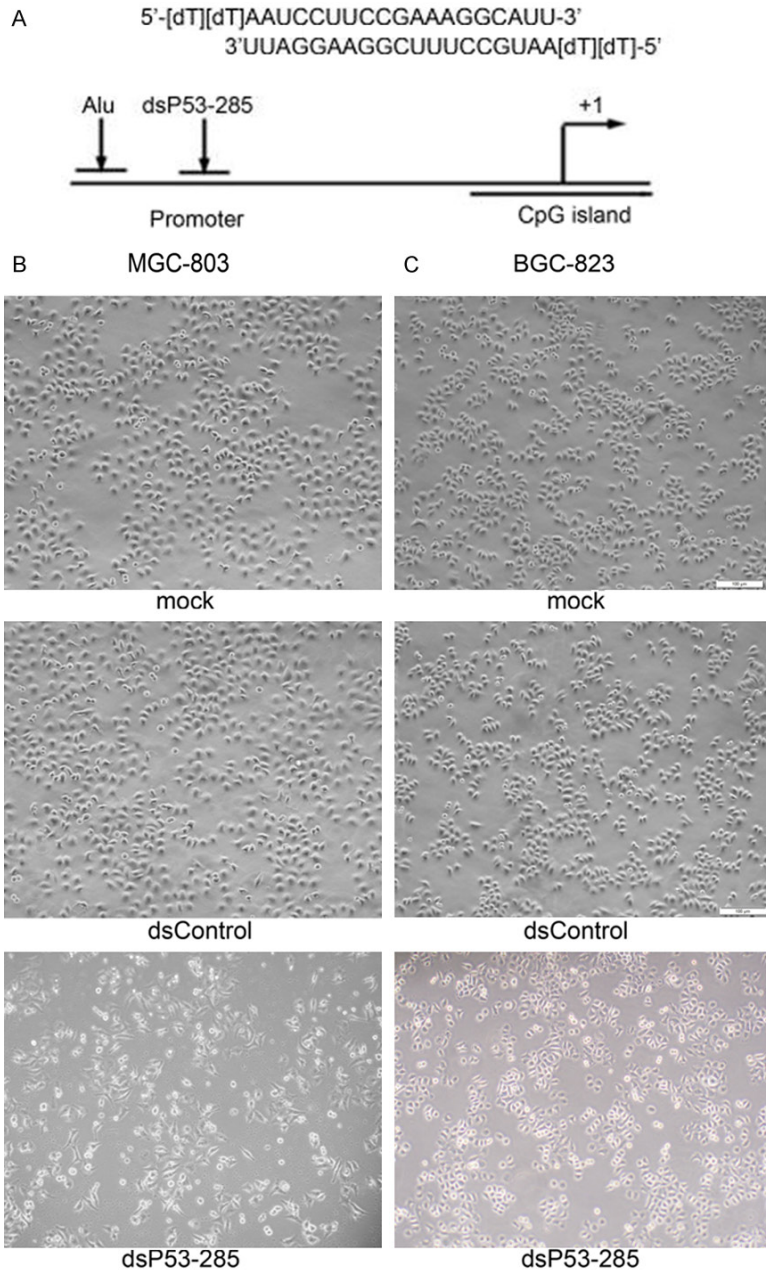

Figure 1. dsP53-285 induced the reactivation of P53 in MGC-803 and BGC-823 gastric cancer cells. The reactivation was triggered by the specific binding between dsP53-285 and the target promoter. The cell morphology was changed after the transfection. A. A schematic representation of the P53 gene promoter and the binding site. B. The MGC-803 gastric cancer cell morphology was changed - MGC-803 cells were treated with dsP53-285 at a concentration of 50 nM for 72 h. Then some were collected for the following experiments, and some were left for the next transfection. After the fifth generation, we could see the change. C. The BGC-823 gastric cancer cell morphology was changed as well. Compared with the control groups, the gastric cancer cells transfected by saRNA looked irregular and had more pseudopod, and the shrinkage of the nuclei was more marked and there were more fragments in the cytoplasm; in addition, there was more cell lysis in the dsP53-285 group.

enhanced chemiluminescence (ECL) system (Pierce Biotechnology Inc., Rockford, IL, USA).

CCK-8 cell proliferation assay

A CCK-8 assay was performed to assess the effect of dsP53-285 on cell proliferation. The transfected MGC-803 and BGC-823 cells were seeded in a 96-well plate at a density of 1.0×10^3 well^{-1} and 1.5×10^3 well^{-1}, respectively, for the CCK-8 proliferation assay. Cell growth was measured at 24 h, 48 h, 72 h, 96 h and 120 h. At each time point, 12 μl of CCK-8 was added to each well containing MGC-803 cells, and 15 μl of CCK-8 for BGC-823; then they were incubated at 37°C for an additional 4 h. Half of the MGC-803 and BGC-823 cells that were transfected with dsP53-285, were also transfected with siP53 to eliminate the off-target effect. The absorbance at 490 nm was determined by using a microreader. All experiments were performed in triplicate.

Scratch-healing assay

MGC-803 GC cells were seeded into six-well plates at a density of 1.0×10^5 cells per well, and for BGC-823, the density was 1.5×10^5 per well. After overnight incubation, the cells were transfected with 50 nmol/l dsP53-285 or dsControl RNA or dsP53-285-siP53 for 72 h. When the cells reached full confluence, they were scratched with a 200 μL sterile pipette tip, then washed with sterilized PBS and cultured in a serum-free medium.

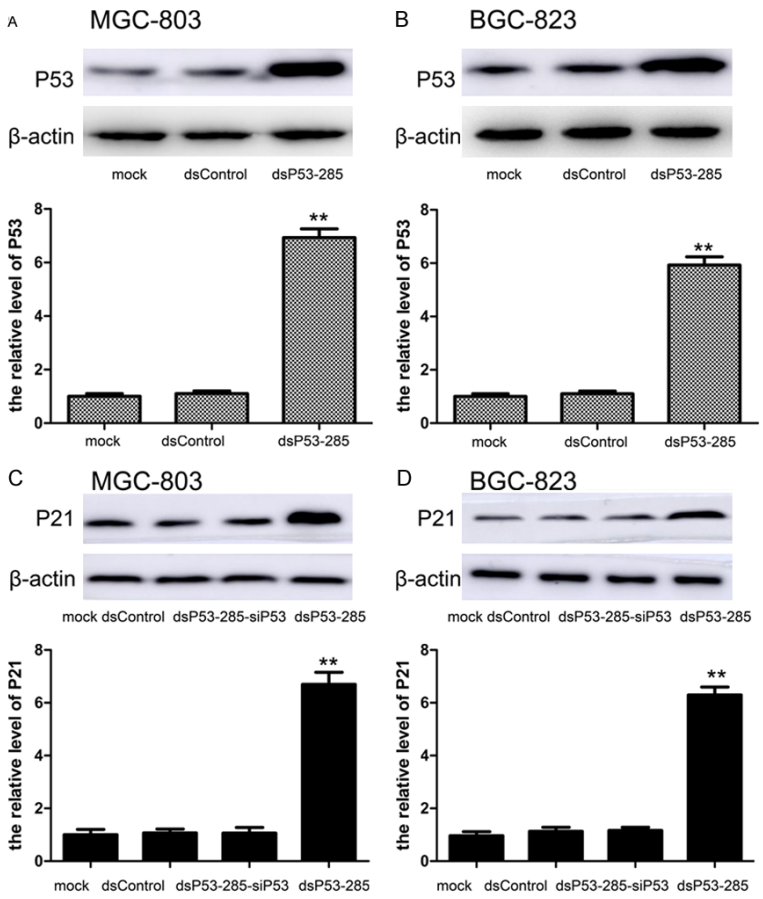

Figure 2. dsP53-285 induced p53 and p21 protein up-regulation in MGC-803 and BGC-823 cells. A, C. The expression of the p53 and p21 proteins was assessed by Western blot. dsP53-285 caused about a 6.5-fold increase expression of p53 protein and a 6.3-fold increase expression of p21 protein in MGC-803 cells. B, D. They showed the expression of p53 and p21 in BGC-823 cells. DsP53-285 caused about a 5.8-fold increased expression of p53 and a 6.2-fold increased expression of p21. (*indicates P<0.05, and **indicates P<0.01 compared to control groups.) The p53 and p21 expression levels were normalized to β-actin and the results were presented as means ± SD of the three independent experiments.

The wound area caused by the scratch was monitored every 12 h, and three random non-overlapping areas in each well were pictured at 0 h, 18 h and 36 h. The wound width between the two linear regions was used to assess the migration ability of the GC cells.

Statistical analysis

All the data were expressed as means ± standard deviation (SD, n≥3). Comparison of the means was performed by Student's t-test and one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests using SPSS (version 17.0) and Prism5 (GraphPad) Software. P<0.05 was considered statistically significant.

Results

dsP53-285 induced the reactivation of P53 in MGC-803 and BGC-823 gastric cancer cells

The reactivation was triggered by the specific binding between dsP53-285 and the target promoter. The cell morphology was changed after the transfection. A schematic representation of the P53 gene promoter and the binding site is shown in **Figure 1A**. The MGC-803 gastric cancer cell morphology was changed. MGC-803 cells were treated with dsP53-285 at a concentration of 50 nM for 72 h. Then some were collected f or the following experiments, some were left for the next transfection. After the fifth generation, we would see the change (**Figure 1B**). The BGC-823 gastric cancer cell morphology was changed. It was treated as above (**Figure 1C**).

dsP53-285 induced p53 and p21 up-regulation in MGC-803 and BGC-823 cells

The expression of the p53 and p21 proteins was assessed by Western blot. DsP53-285 caused about a 6.5-fold increase expression of p53 protein and a 6.3-fold increase expression of p21 protein in MGC-803 cells (**Figure 2A**, **2C**). They showed the expression of p53 and p21 in BGC-823 cells. DsP53-285 caused about a 5.8-fold increased expression of p53 and a 6.2-fold increased expression of p21 (**Figure 2B**, **2D**). They showed the expression of the P53 and P21 RNA of each group. The dsP53-285 groups were higher than the other control groups (**Figure 3A**, **3D**) (P<0.01). The dsP53-285-siP53 complex was used to exclude the off-target effect. The p53 and p21 expression levels were normalized to β-actin and the results were presented as means ± SD of three independent experiments. (*indicates P<0.05, and **indicates P<0.01 compared to the control groups).

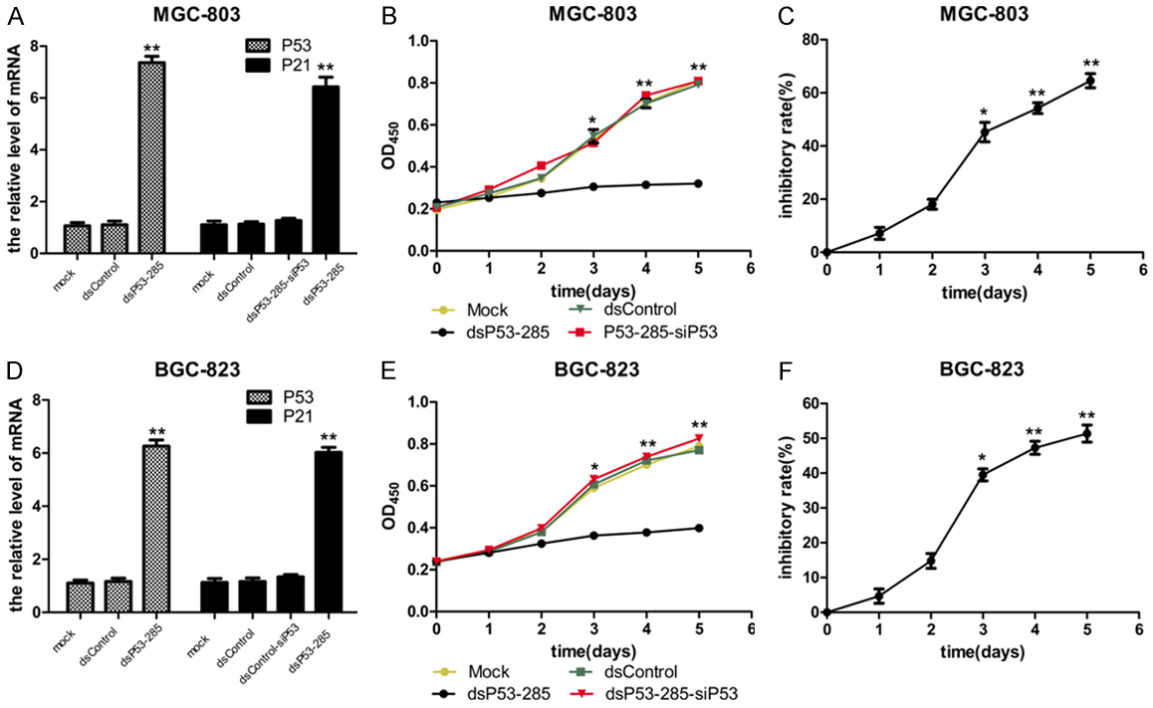

Figure 3. A, D. dsP53-285 induced p53 and p21 mRNA up-regulation in MGC-803 and BGC-823 cells. The dsP53-285-siP53 groups were designed to eliminate the off-target effect. B, C. The growth and proliferation of the gastric cancer cell, MGC-803, was inhibited by dsP53-285; 72 h later after transfection, we observed the significant difference of the relative inhibitory rate. E, F. The BGC-823 cells showed a similar phenomenon. (*indicates P<0.05, and **indicates P<0.01).

dsP53-285 inhibited the growth and proliferation of GC cells

The CCK-8 assay was used to assess the inhibitory effect. In this assay, the OD_{450} was used to assess the growth of the cells. On the third day, we observed a significant difference between the dsP53-285 group and the other groups (P<0.05). The curve showed the growth of the dsP53-285 groups of MGC-803 was inhibited. And we observed the difference on the third day (**Figure 3B**, **3C**) (P<0.05). The curve showed BGC-823 cells (**Figure 3E**, **3F**). The results were presented as means ± SD of three independent experiments. (*indicates P<0.05, and **indicates P<0.01 compared to the control groups).

dsP53-285 inhibited the migration of GC cells

The scratch-healing assay was used to assess the migration ability of each group. We found that the migration ability of the dsP53-285 group was inhibited significantly compared with other groups (P<0.01). The migration ability of MGC-803 cells was inhibited. In dsP53-285 groups of MGC-803, the cell migration number was less; and the distance was much shorter (**Figure 4**). For BGC-823, it was similar (**Figure 5**). (*indicates P<0.05, and **indicates P<0.01).

Discussion

In 2012, of all the cancer cases diagnosed, gastric cancer accounted for 6.8%; and of all cancer-related deaths, gastric cancer accounted 8.8% [1]. Gastric cancer is a heterogeneous disease, and there are many ways to treat it, including surgical resection and chemoradiotherapy, or both. However, survival is poor under the current therapeutic regimens [16]. Therefore, there is a great need to find a more effective treatment to improve the therapeutic outcomes. A new biomarker, saRNA, comes to mind.

Since RNAa was brought up, the mechanism and principal were gradually revealed. Some studies showed that Ago2 could stimulate transcription through targeting the RITA complex to promoters under saRNA guidance; add-

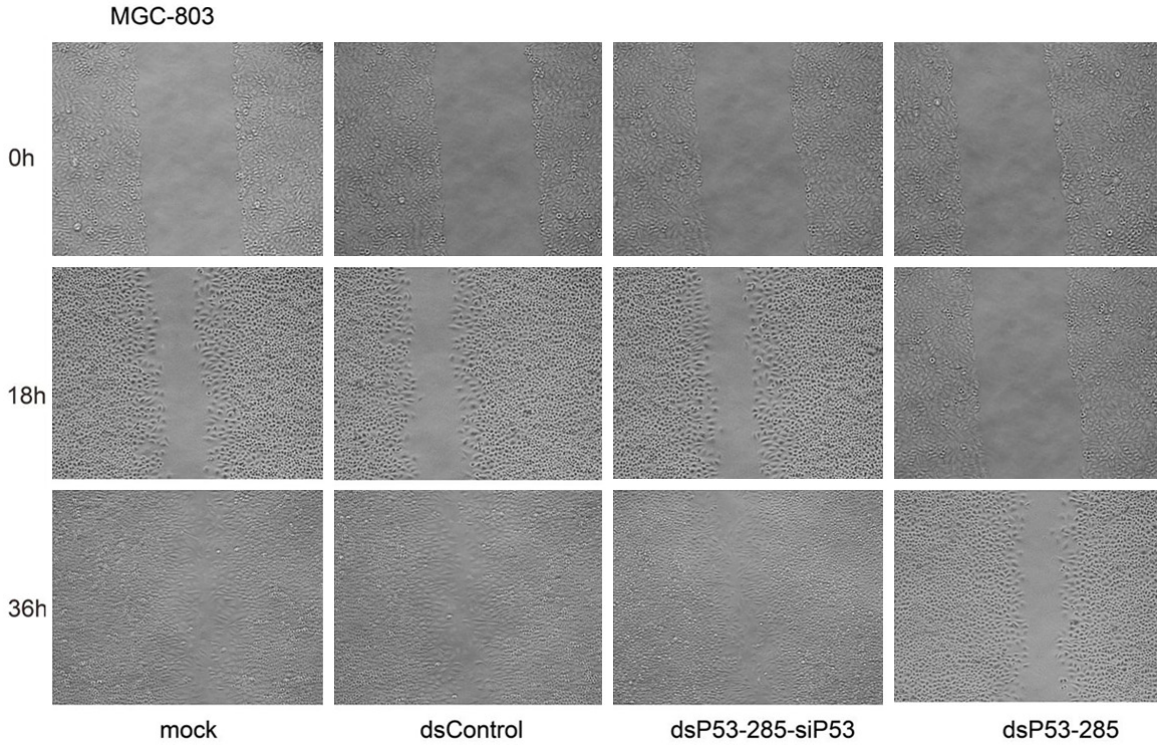

Figure 4. dsP53-285 inhibited the migration of MGC-803 cells. The number of migration cells was significantly smaller in the dsP53-285 transfection groups, and the migration distance was significantly shorter in the dsP53-285 transfection groups. (*indicates P<0.05, and **indicates P<0.01).

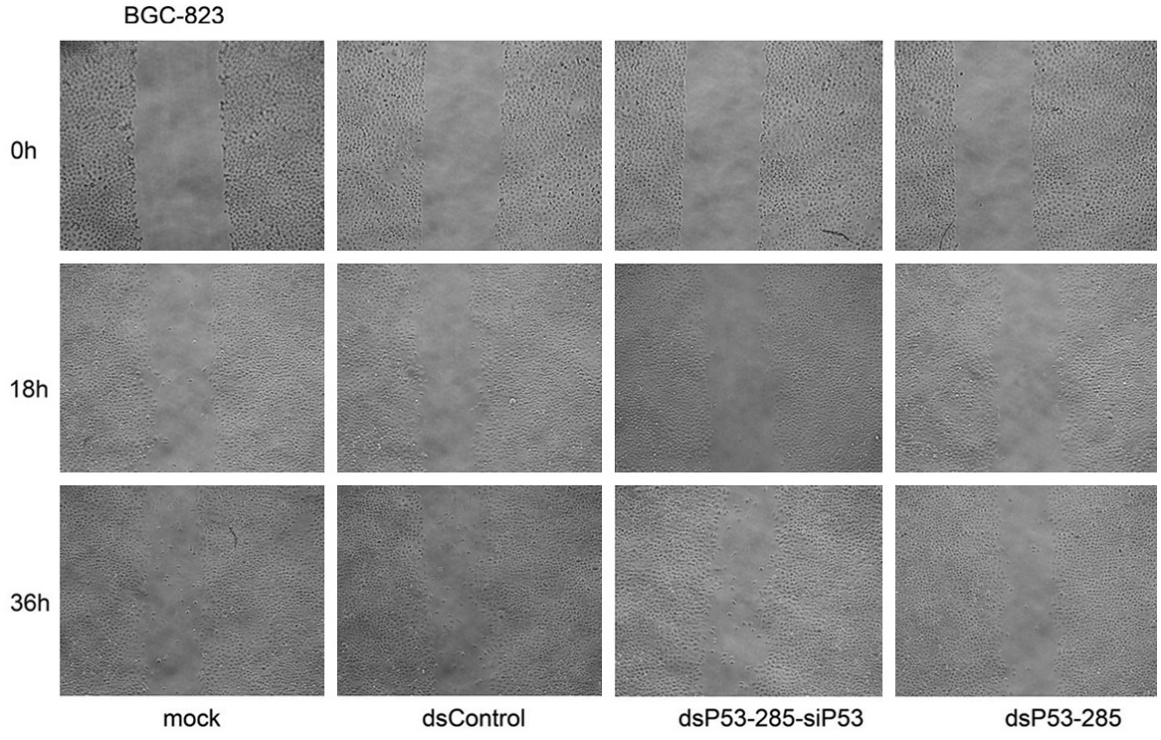

Figure 5. dsP53-285 inhibited the migration of BGC-823 cells. The number of migration cells was significantly smaller in the dsP53-285 transfection groups, and the migration distance was significantly shorter in the dsP53-285 transfection groups. (*indicates P<0.05, and **indicates P<0.01).

itional studies illustrated that the activation could be triggered by the interaction between saRNA and heterogeneous nuclear ribonucleoprotein A2/B1; the methylation of the gene promoter is associated with RNAa [21, 22]. SaRNA has proven to be feasible in many genes in different cancers, including E-cadherin, VEGF, p21, PAR, WT1, HIC1 and VEZT [5, 6, 13, 21-24]. Also, Ren and Kang proved the feasibility of RNAa in animals, and they found that tumor growth could be inhibited by saRNA which is formulated into lipid nanoparticles [25, 26]. Moreover, compared to the traditional therapeutic tools, saRNA is more beneficial due to its low toxicity and high specificity and efficacy [5].

The P53 gene, which is known as the guardian of the genome, could encode the p53 protein, a transcription factor. The p53 protein could upregulate the expression of the broad-acting, cyclin-dependent kinase inhibitor, the P21 gene, and then promote cell cycle arrest at the G1 phase; besides, the P53 gene could regulate cell apoptosis through a different pathway, mainly including the mitochondrial pathway and the death receptor pathway [27, 28].

In this study, we transfected gastric cancer cells with dsP53-285, dsP53-285-siP53, ds-Control, or lipofectamine2000, respectively. That was the most important part of the experiment, and usually the transfection was at a concentration of 50 nM. When it was at a lower concentration, it was less effective, and when at a higher concentration, it was of less value [5, 8]. Then we verified the practicability of RNAa through different methods. The Western blot and RT-qPCR showed that dsP53-285 could up-regulate the expression of the P53 gene, and the downstream receptor, p21, was upregulated as a result. The CCK-8 assay proved that dsP53-285 could inhibit the growth of gastric cancer cells and indicated that the reactivation effect was in a time-course manner, and that was in accordance with previous studies [5, 6]. Interestingly, we found that in the dsP53-285-siP53 group, the proliferation was a little fast; however, that made no statistical sense, and that might be caused by the stronger interference of siP53 or the minor different transfection. Then the scratch-healing assay verified that the migration ability of gastric cancer cells was inhibited by dsP53-285. The dsP53-285-siP53 group was mainly designed to exclude the off-target effect. Certainly, our study was not perfect, and it has its own limits. We just confirmed the reactivation feasibility of dsP53-285 in gastric cancer, rather than perfect the design rule of saRNA that was a common problem for us all. Besides, we didn't illuminate the mechnism of RNAa in a more direct way. And the animal experiment was needed to further prove the feasibility of RNAa. Therefore, in our subsequent experiments, we will explore more details about RNAa.

In general, the mechanism of RNAa and the saRNAs designed rules were ambiguous, but the problems could be solved by using bioinformatics software to some extent and corresponding experiments, and that was the most effective method. Therefore, RNAa, a promising therapeutic tool for cancer treatment, is worthy of further study.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China No.81572355 and National Foundation of Shandong Province under Grant number ZR2015PH024.

Disclosure of conflict of interest

None.

Address correspondence to: Yanbing Zhou, Department of General Surgery, Affiliated Hospital of Qingdao University, Qingdao 266021, Shandong, China. Tel: +86-18678896912; E-mail: zhouyanbing0901@163.com

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-86.

[2] Kilic L, Ordu C, Yildiz L, Sen F, Keskin S, Ciftic R, Pilanci KN. Current adjuvant treatment modalitie for gastric cancer: from history to the future. World J Gastrointest Oncol 2016; 8: 439-449.

[3] Jou E and Rajdev L. Current and emerging therapies in unresectable and recurrent gastric cancer. World J Gastroenterol 2016; 22: 4812-23.

[4] Cunningham D, Okines AF, Ashley S. Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 2010; 362: 858-9.
[5] Li LC, Okino ST, Zhao H, Pookot D, Place RF, Urakami S, Enokida H and Dahiya R. Small dsRNAs induce transcriptional activation in human cells. Proc Natl Acad Sci U S A 2006; 103: 17337-42.
[6] Janowski BA, Younger ST, Hardy DB, Ram R, Huffman KE and Corey DR. Activating gene expression in mammalian cells with promoter-targeted duplex RNAs. Nat Chem Biol 2007; 3: 166-73.
[7] Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microrna can upregulate translation. Science 2007; 318: 1931-1934.
[8] Yang B, Duan X, Wu W, Ji W, Wu W, Zhong W, Zhao Z, Li S, Liu Y and Zeng G. Induction of TRPV5 expression by small activating RNA targeting gene promoter as a novel approach to regulate cellular calcium transportation. Life Sci 2014; 114: 70-6.
[9] Place RF, Li LC, Pookot D, Noonan EJ and Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. Proc Natl Acad Sci U S A 2008; 105: 1608-13.
[10] Portnoy V, Huang V, Place RF and Li LC. Small RNA and transcriptional upregulation. Wiley Interdiscip Rev RNA 2011; 2: 748-60.
[11] Wang X, Wang J, Huang V, Place RF and Li LC. Induction of NANOG expression by targeting promoter sequence with small activating RNA antagonizes retinoic acid-induced differentiation. Biochem J 2012; 443: 821-8.
[12] Yue X, Schwartz JC, Chu Y, Younger ST, Gagnon KT, Elbashir S, Janowski BA and Corey DR. Transcriptional regulation by small RNAs at sequences downstream from 3' gene termini. Nat Chem Biol 2010; 6: 621-629.
[13] Huang V, Qin Y, Wang J, Wang X, Place RF, Lin G, Lue TF, Li LC. RNAa is conserved in mammalian cells. PLoS One 2010; 5: e8848.
[14] Braasch DA and Corey DR. Novel antisense and peptide nucleic acid strategies for controlling gene expression. Biochemistry 2002; 41: 4503-10.
[15] Schwartz JC, Younger ST, Nguyen NB, Hardy DB, Monia BP, Corey DR and Janowski BA. Antisense transcripts are targets for activating small RNAs. Nat Struct Mol Biol 2008; 15: 842-8.
[16] Charalampakis N, Economopoulou P, Kotsantis I, Tolia M, Schizas D, Liakakos T, Elimova E, Ajani JA and Psyrri A. Medical management of gastric cancer: a 2017 update. Cancer Med 2018; 7: 123-133.
[17] Mello SS and Attardi LD. Deciphering p53 signaling in tumor suppression. Curr Opin Cell Biol 2018; 51: 65-72.
[18] Jang SJ, Park YW, Park MH, Lee JD, Lee YY, Jung TJ, Kim IS, Choi IY, Ki M, Choi BY, Ahn MJ. Expression of cell-cycle regulators, cyclin E and p21WAF1/CIP1 potential prognostic markers for gastric cancer. Eur J Surg Oncol 1999; 25: 157-63.
[19] Xi YG, Ding KY, Su XL, Chen DF, You WC, Shen Y and Ke Y. p53 polymorphism and p21WAF1/CIP1 haplotype in the intestinal gastric cancer and the precancerous lesions. Carcinogenesis 2004; 25: 2201-6.
[20] Mizokami K, Kakeji Y, Oda S and Maehara Y. Relationship of hypoxia-inducible factor 1alpha and p21WAF1/CIP1 expression to cell apoptosis and clinical outcome in patients with gastric cancer. World J Surg Oncol 2006; 4: 94.
[21] Portnoy V, Lin SH, Li KH, Burlingame A, Hu ZH, Li H and Li LC. saRNA-guided Ago2 targets the RITA complex to promoters to stimulate transcription. Cell Res 2016; 26: 320-35.
[22] Hu J, Chen Z, Xia D, Wu J, Xu H and Ye ZQ. Promoter-associated small double-stranded RNA interacts with heterogeneous nuclear ribonucleoprotein A2/B1 to induce transcriptional activation. Biochem J 2012; 447: 407-16.
[23] Xie D, Shang L, Peng L and Li L. Up-regulation of VEZT by small activating RNA inhibits the proliferation, invasion and migration of gastric cancer cells. Biochem Biophys Res Commun 2016; 482: 542-548.
[24] Zhao F, Pan S, Gu Y, Guo S, Dai Q, Yu Y and Zhang W. Reactivation of HIC-1 gene by saRNA inhibits clonogenicity and invasiveness in breast cancer cells. Oncol Lett 2015; 9: 159-164.
[25] Ren S, Kang MR, Wang J, Huang V, Place RF, Sun Y and Li LC. Targeted induction of endogenous NKX3-1 by small activating RNA inhibits prostate tumor growth. Prostate 2013; 73: 1591-601.
[26] Kang MR, Yang G, Place RF, Charisse K, Epstein-Barash H, Manoharan M and Li LC. Intravesical delivery of small activating RNA formulated into lipid nanoparticles inhibits orthotopic bladder tumor growth. Cancer Res 2012; 72: 5069-79.
[27] Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell 2002; 2: 103-12.
[28] Li N, Xie C, Lu NH. p53, a potential predictor of Helicobacter pylori infection-associated gastric carcinogenesis? Oncotarget 2016; 7: 66276-66286.