

Research article

**THE KNOCKDOWN OF c-myc EXPRESSION BY RNAi INHIBITS
 CELL PROLIFERATION IN HUMAN COLON CANCER HT-29 CELLS
in vitro AND *in vivo***

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Abstract: We investigated the effects of RNA interference-mediated silencing of the c-myc gene on cellular proliferation and apoptosis in human colon cancer HT-29 cells *in vitro* and *in vivo*. A small interfering RNA (siRNA) targeting c-myc was designed, the DNA template was synthesized, and the siRNA was obtained by *in vitro* transcription. After siRNA transfection into HT-29 and human neuroblastoma IMR-32 cells with Lipofectamine 2000TM, the proliferation of the HT-29 and IMR-32 cells was assessed via 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetry, and Hoechst 33258 staining was used to observe cell apoptosis. Following gene transfer to HT-29 cells, the expression of c-myc mRNA was examined via reverse transcription polymerase chain reaction, and the level of the protein via Western blot assay. Growth curves were constructed and *in vivo* experiments were performed on nude mice to assess the effects of c-myc silencing on tumor growth. The c-myc expression in the tumor tissue was measured by reverse transcription polymerase chain reaction and subsequently by immunohistochemistry. Our paper demonstrates that the delivery of siRNA directed against c-myc not only efficiently down-regulated the expression of c-myc, inhibited the proliferation of HT-29 cells and induced apoptosis *in vitro*, but also suppressed the growth of colon cancer cells *in vivo*.

Key words: c-myc, Cell proliferation, HT-29, siRNA

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Abbreviations used: DMEM – Dulbecco's modified Eagle's medium; FBS – fetal bovine serum; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; MTT – 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; PBS – phosphate-buffered saline; RT-PCR – reverse transcription polymerase chain reaction; SDS – sodium dodecyl sulphate; siRNA – short interfering RNA

INTRODUCTION

Colon cancer is one of the most common cancers worldwide, and considerable progress has been made through molecular and biological research in identifying the mechanisms of its tumorigenesis [1]. In this regard, several investigations have been directed toward uncovering alterations in the gene structure, gene expression, or activity of gene products associated with this prevalent disease [2]. The members of the myc proto-oncogene family, c-, L-, and N-myc, are thought to be central regulators of cell growth, and deregulated myc expression is associated with many cancers, including colon cancer [3-5]. c-myc is believed to participate in most aspects of cellular function, including replication, growth, metabolism, differentiation, and apoptosis [6-8]. A frequent genetic abnormality seen in colon cancer is the elevated expression of c-myc [4, 9]. The importance of c-myc expression in colon cancer has been demonstrated in both studies of transgenic mice and clinical research [10, 11]. Clinical studies have also indicated that c-myc is important in the development and progression of colon cancer, since over-expression of the oncogene was found in most colon cancer patients, correlated with poor prognosis [4].

The role of c-myc in colon cancer has been extensively studied for the past decade [12], and specific reduction of its level by genetic means in colon cancer cell lines is believed to be valuable in understanding its sustained malignant phenotype. Thus, in this study, we investigated whether specifically decreasing c-myc expression in HT-29 cells in which this gene was overexpressed could result in the inhibition of cell growth and induction of cell apoptosis *in vitro* and *in vivo*. RNA interference (RNAi) directed against c-myc was used for this purpose.

RNAi is a natural post-transcriptional, sequence-specific gene regulatory cell mechanism that controls gene expression by degrading the corresponding endogenous mRNA. The mediators of this process are small, double-stranded RNAs (21 to 23 bp in length), the so-called short interfering RNAs (siRNAs). These siRNAs are processed from larger dsRNAs through the Dicer enzyme, forming the RISC complex (RNA-induced silencing complex); this complex guides the siRNA to the corresponding homologue mRNA and cleaves it at the binding site region [13].

Hoping to apply siRNA to inhibit c-myc expression in human colon cancer cells, we designed two pairs of siRNA according to the c-myc sequence. They were synthesized by T7 RNA polymerase transcription *in vitro*. We then evaluated the inhibitory activity of siRNA by transfecting cells via Lipofectamine 2000TM. The siRNA targeting c-myc determined whether this technique could be used for the specific inhibition of oncogene over-expression and whether this inhibition resulted in anti-tumor effects. This study could therefore demonstrate that specific downregulation of c-myc by RNAi is sufficient to inhibit the growth of HT-29 cells *in vitro* and *in vivo*.

MATERIALS AND METHODS

Short-interfering RNA (siRNA) design

The Promega software analysis system was employed to select siRNA directed against c-myc mRNA (GenBank: ACCESSION: NM-002467), and a BLAST search verified the absence of significant homology with other human genes. The siRNA sequences directed against c-myc mRNA were likewise selected according to their BLAST search score and GC content (40-60%). Two pairs of DNA oligonucleotides for *in vitro* transcription were designed and ordered from Sangon Bio Corp. The DNA template for the *in vitro* transcription of the siRNA was a short duplex oligonucleotide that consisted of the target sequence and the T7 RNA polymerase promoter sequence, as well as six extra nucleotides upstream of the minimal promoter sequence to allow for more efficient T7 RNA polymerase binding. Additional adenine-adenine nucleotides were also added to the 5' end of the oligonucleotide to allow the addition of two uridine 3' overhangs in the siRNA strands. Two oligonucleotides were annealed to generate separate templates for the synthesis of each siRNA strand. After the separate short RNA strands were synthesized, they were annealed to form siRNA. We employed the "T7RiboMAX™ Express RNAi System" kit (Promega, USA) to synthesize the siRNA, as well as one scrambled siRNA (used as a negative control) with the following sense and antisense sequences: siRNA (1764-1782 bp), 5'-GCCACAGCAUACAUCCUGUUU-3' (sense), 5'-ACAGGAUGUAUGCUGUGGCUU-3' (antisense); scrambled siRNA (siRNAsc), 5'-GACUUCAUAAGGCGCAUGCUU-3' (sense), 5'-GCAUGCGCCUUAUGAAGUCUU-3' (antisense).

Cell culture

ATCC HT-29 colon cancer and IMR-32 human neuroblastoma cell lines were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% fetal bovine serum (FBS), 50 units/ml penicillin, and 50 µg/ml streptomycin. The cells were kept in culture at 37°C and 5% CO₂, and the medium was changed every three days. To keep the cells at optimal proliferating conditions, they were passaged at 80% confluence and seeded at 20% confluence.

Transient siRNA transfection

The HT-29 and IMR-32 cells were trypsinized and diluted to the appropriate concentration (8×10^7 cell/l) in fresh, antibiotic-free medium. Transfections of siRNA or siRNAsc were performed with Lipofectamine 2000™ (Promega) according to the manufacturer's instructions at a final ratio of 1:2 or 1:3. Next, the cells in the blank control group were treated with OPTI-MEM medium alone, while the cells in the lipofectamine control group were treated with Lipofectamine 2000™-OPTI-MEM. The medium was changed for the normal culture medium 6 h after transfection.

MTT assay

In a 96-well plate, a total of 8×10^3 HT-29 or IMR-32 cells were seeded in each well and allowed to attach for 18 h. The cells were later treated with 200 nmol/l siRNA or 200 nmol/l siRNAscr. At 24, 48, and 72 h after transfection, 10 μ l 10 mg/ml MTT was added to the cells in each well. The cells were incubated for 4 h, after which 100 μ l DMSO was added, and the cells were allowed to lyse for 15 min. All of the analyses were carried out in triplicate. The optical density was determined at 492 nm with a microculture plate reader. To determine the inhibitor rate, the absorbance values were then normalized to the values obtained from the blank control group cells.

Apoptotic cell morphology observation

The HT-29 or IMR-32 cells were seeded in 24-well plates with glass slides on the bottom of the wells, and treated with 200 nmol/l siRNA for 48 h. After the slides had been gently washed with cold PBS, they were fixed with 4% polyformaldehyde for 1 h and washed three times with PBS. The resulting cells were stained with 0.5 ml Hoechst 33258 (10 μ g/ml) at 37°C for 10 min in a dark room. The apoptotic features of cell death were established by staining the cell nuclei with the DNA-binding fluorochrome Hoechst 33258 and assessing the chromatin condensation by fluorescence microscopy. In each group, six microscopic fields were randomly selected and five hundred cells were counted. The apoptotic cell level was then calculated as the percentage of apoptotic cells over the total number of blue fluorescent protein-positive cells.

RT-PCR analysis: the mRNA levels of c-myc and N-myc

Total RNA extracted with Trizol reagent was prepared from HT-29 cells (2×10^6) transfected with 200 nmol/l siRNA for 48 h. Subsequently, the RNA precipitate was centrifuged and dissolved in 20 μ l of RNase-free water. UV spectrophotometer analysis at 260 nm and electrophoresis detection revealed the good quality of the purified RNA. The total RNA measured at 1 μ g was reverse transcribed to synthesize cDNA at 42°C for 1 h; this cDNA was then subjected to PCR amplification with specific primers in 25- μ l mixtures. The primers for c-myc were 5'-CTCTCAACGACAGCAGCCCG-3' (forward) and 5'-CCAGTCTCAGACCTAGTGGA-3' (reverse) with an amplification product of 250 bp. The primers for N-myc were 5'-CTGTCGCCCAGCCGTGGCTT-3' (forward) and 5'-GGCCGTGCTGCAGCTTCGCG-3' (reverse) with an amplification product of 232 bp. GAPDH served as an internal control. The primers for GAPDH were 5'-CGTGGAAAGGACTCATGACCA-3' (forward) and 5'-TCCAGGGGTCTTACTCCTTG-3' (reverse), with an amplification product of 512 bp. c-myc was amplified through 35 cycles of denaturing at 94°C for 45 s, annealing at 56°C for 1 min, and extending at 68°C for 1 min, with a further 10 min extension. N-myc PCR underwent 35 cycles with denaturing at 94°C for 45 s, annealing at 64°C for 1 min, and extending at 72°C for 1 min, with a further 10 min extension. Next, the PCR products were electrophoresed on 2%

agarose gel, stained with ethidium bromide, and observed by UV irradiation. The digital images were analyzed with Tanon image analysis software. The results were expressed as the ratio of the expression level of c-myc and N-myc over GAPDH.

Western blot analysis

Following treatment with 200 nmol/l siRNA for 48 h, HT-29 cells attached to culture dishes were trypsinized and washed in PBS. Briefly, 50- μ g samples of protein were subjected to 10% standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), with prestained molecular weight markers run in parallel to identify c-myc proteins. Afterward, the resolved proteins were transferred to nitrocellulose membranes. The antibodies and dilutions used included anti-c-myc (9E10; 1:1000 dilution; Santa Cruz) and anti- β -actin (AC-15; 1:5000 dilution; Sigma). After extensive washing, the membranes were incubated with anti-mouse IgG-horseradish peroxidase conjugate antibody (Zhongshan Company) for 1 h at room temperature, and developed with a Luminol chemiluminescence detection kit (Santa Cruz). Membranes probed for c-myc were reprobated for β -actin to normalize for loading and/or quantification errors and to allow comparisons of target protein expression. The protein expression was quantified with a Gel EDAS analysis system (Cold Spring USA Corporation) and Gel-Pro Analyzer 3.1 software (Media Cybernetics).

Tumor growth in nude mice

Five-week old female outbred Balb/c-nu/nu mice were purchased from Shanghai SLAC Laboratory Animal Co. Ltd., and kept under specific pathogen-free conditions. Using a 24-gauge needle, a total of 3.0×10^6 HT-29 cells in 0.3 ml of serum-free DMEM medium were inoculated into the mid-dorsum of 6-week old athymic nude mice. After three weeks, when the tumors had reached an average volume of 50-60 mm³, the tumor-bearing nude mice were treated with 0.1 ml 10 μ mol/l c-myc siRNA or siRNAsc_r used after dilution with PBS. The blank controls were injected with 0.1 ml PBS. Each therapeutic reagent was injected into the tumors every three days after the first injection, as indicated. The tumor diameters were measured at regular intervals with digital calipers, and the tumor volume in mm³ was calculated using the following formula: volume = (width)² × length/2. A tumor growth curve was then constructed, and the data was presented as the means \pm S.E. Three days after the termination of s.c. tumor treatment, the mice were sacrificed by cervical dislocation, and their tumors were removed. The tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned, stained with haematoxylin and eosin, and observed through microscopy.

Histological analysis of the tumor xenografts

Paraffin-embedded tissue sections were dewaxed and incubated with peroxidase solution at room temperature for 10 min, and tumor antigenicity was restored by microwave treatment or trypsin digestion. Tumor sections were next incubated

with non-immune sera at room temperature for 10 min, and then incubated with primary rabbit antibodies against c-myc (c-19; Santa Cruz) at 4°C overnight. Next, biotin antibody was added to the sections, which were then incubated at 37°C for 30 min, after which streptavidin-peroxidase solution was added, followed by another incubation for 10 min at room temperature. The peroxidase activity was assessed using diaminobenzidine (Sigma), and the nuclei were counterstained with haematoxylin.

RT-PCR analysis: the mRNA levels of c-myc in the tumor xenografts

The tumor total RNA was extracted with Trizol reagent. The RNA precipitate was centrifuged and dissolved with 20 µl of RNase-free water. A UV spectrophotometer analysis at 260 nm and electrophoresis detection revealed the good quality of the purified RNA. The total RNA measured at 1 µg was reverse transcribed to synthesize cDNA at 42°C for 1 h, following which the cDNA was subjected to PCR amplification with specific primers in 25-µl mixtures. PCR was performed as per the previous description.

Statistical analysis

The data is given as the means ± S.D. of the indicated number of separate experiments. The one-way analysis of variance was performed for multiple comparisons. If there was a significant variation between the treatment and control groups, Student's t-test was used to compare the mean values. A value of $P < 0.05$ was considered significant.

RESULTS

The proliferation of HT-29 and IMR-32 cells after the siRNA transfection of c-myc

Previous studies have shown that c-myc is significant in cellular proliferation and cell growth [6, 8, 14]. Thus, the high levels of c-myc seen in colon tumors give a probable growth advantage. Our data showed that in the group treated with c-myc siRNA, the A value and the cell inhibitor rates were different from those of the lipofectamine control group at any given time ($P < 0.01$), and there were significantly reduced growth rates of HT-29 cells, with 40-60% decreases between 24 and 72 h after siRNA transfection. The inhibitory effect was stable at 48 to 72 h after siRNA transfection. By contrast, there were significantly less inhibitory effects on HT-29 cells in the c-myc siRNA-free group (blank control) and the lipofectamine control group ($P > 0.05$). Under the same conditions, none of our siRNA formulations inhibited the proliferation of the c-myc negative cell line IMR-32. To demonstrate the sequence specificity of these effects, scrambled-sequence siRNA (siRNAsc) were also delivered into HT-29 and IMR-32 cells. Little or no growth inhibition was observed for siRNAsc (Fig. 1).

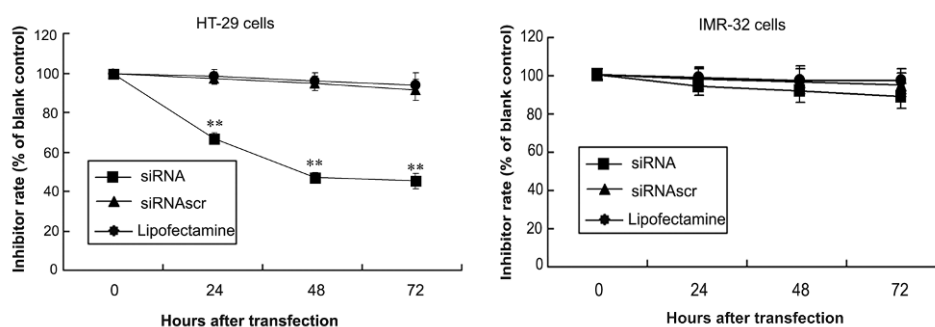


Fig. 1. The proliferation of HT-29 and IMR-32 cells after the siRNA transfection of c-myc. The cells were treated with siRNA, siRNAsc, or lipofectamine (without siRNA), or were left untreated as the control (blank), as described in the Materials and Methods section. The data was expressed as a percentage of the blank control. The data is shown as the means \pm S.D. of three independent experiments. c-myc siRNA markedly inhibited the growth of HT-29 cells, but had no effect on IMR-32 cells. ** $P < 0.01$, vs. lipofectamine control group.

The morphology of the apoptotic HT-29 cells

An apoptotic cell has special morphological features such as cell shrinkage, chromatin condensation, margination, and apoptotic body formation. Following the treatment with Hoechst 33258 in this study, the apoptotic bodies or nuclear fragments were stained deep blue, and the normal cells were stained light blue, while the boundaries between the cytoplasm and nuclei of the necrotic cells were unclear because of organelle swelling and lysis. We found that 21.7% of HT-29 cells transfected with siRNA underwent apoptosis compared with 4.8% of the siRNAsc control group (Tab. 1). Under the same conditions, none of our siRNA formulations induced apoptosis of the c-myc negative cell line IMR-32 (Fig. 2).

Tab. 1. The effect of siRNA on apoptosis in HT-29 and IMR-32 cells ($\bar{x} \pm s$, $n = 3$).

Group	Apoptotic cells (%)	
	HT-29 cells	IMR-32 cells
siRNA group	21.74 \pm 2.31**	5.19 \pm 0.76
siRNAsc group	4.83 \pm 0.91	5.12 \pm 0.87
Lipofectamine group	5.34 \pm 1.28	4.97 \pm 0.82
Blank control group	4.69 \pm 0.85	4.58 \pm 0.53

The level of apoptotic cells was then calculated as the percentage of apoptotic cells over the total blue fluorescent protein-positive cells. The means and S.D. of three independent experiments are indicated. ** $P < 0.01$ vs. blank control group.

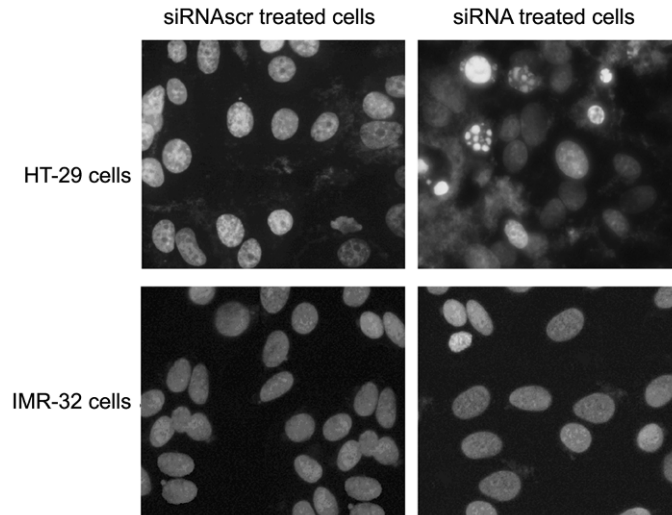


Fig. 2. The induction of apoptosis after the transfection of the siRNA targeting c-myc in HT-29 cells. After the siRNA transfection of the HT-29 and IMR-32 cells for 48 h, Hoechst 33258 staining was done to identify the apoptotic cells ($\times 400$), which show special morphological features. 21.7% of the siRNA-transfected HT-29 cells underwent apoptosis, compared to 4.8% of the cells in the siRNAscrtreated group ($P < 0.01$). No apoptosis was induced in our siRNA formulations of the c-myc negative cell line IMR32, even under similar conditions.

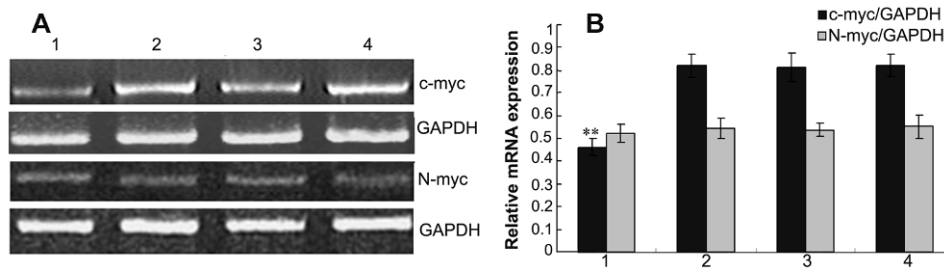


Fig. 3. The c-myc and N-myc mRNA levels in HT-29 cells transfected with siRNA. A – Lane 1, HT-29 cells treated with siRNA; lane 2, HT-29 cells treated with siRNAscrtreated; lane 3, HT-29 cells treated with lipofectamine; lane 4, untreated HT-29 cells (blank control). The mRNA levels of c-myc and N-myc were detected using RT-PCR. The results shown are representative of three independent experiments. B – Quantification of the results from (A). The results were expressed as the ratio of the expression level of c-myc and N-myc over GAPDH. The siRNA transfection reduced the c-myc mRNA level to approximately 50% of the original, while the GRPDH mRNA expression showed no difference between the groups ($P > 0.05$). The N-myc mRNA expression was assessed to examine RNA interference specificity; the expression level of N-myc was unaltered following siRNA transfection.

The effect of c-myc siRNA on the expression of the c-myc mRNA and N-myc mRNA of HT-29 cells

Cells were transfected with siRNA and harvested after 48 h, and c-myc mRNA was examined by RT-PCR. siRNA transfection revealed a reduction of c-myc RNA levels by 50% of the original level (Fig. 3). GRPDH mRNA expression showed no difference between the groups ($P > 0.05$). To investigate the specificity of RNA interference in our study, we also assessed the N-myc mRNA expression, finding that the expression level of N-myc did not change after siRNA transfection (Fig. 3).

The effect of c-myc siRNA on the expression of c-myc protein expression

We next examined the c-myc protein levels from HT-29 cells 48 h after the transfection of siRNA targeting c-myc using Western blot. The c-myc siRNA in the HT-29 cells induced a marked decrease in c-myc synthesis and secretion (Fig. 4) by up to 70% compared with the levels for the blank control group ($P < 0.01$). There was no difference in c-myc protein expression between the cells transfected with siRNAsc, lipofectamine, and the untreated (blank control) cells ($P > 0.05$). In addition, RNAi did not cause a non-specific downregulation of gene expression as determined by the β -actin control (Fig. 4). This revealed the successful transfection of c-myc siRNA into the HT-29 cells and a specific reduction of c-myc protein expression, similar to the results for the c-myc mRNA reduction.

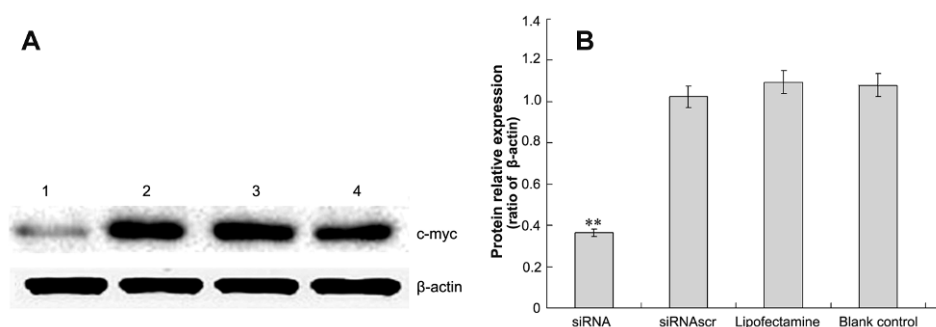


Fig. 4. The reduction in c-myc protein levels after siRNA transfection. A – Lane 1, HT-29 cells treated with siRNA; lane 2, HT-29 cells treated with siRNAsc; lane 3, HT-29 cells treated with lipofectamine; lane 4, untreated HT-29 cells of the blank control group. The total cell lysates were separated by SDS-polyacrylamide-gel electrophoresis and immunoblotted with an antibody against c-myc. The results shown are representative of three independent experiments. B – Quantification of the results from (A). The data was expressed as the ratio of the expression level of c-myc over β -actin. There was approximately a 70% decrease in c-myc synthesis induced by c-myc-siRNA in the HT-29 cells compared to the levels for the blank control group ($P < 0.01$).

Tumor growth reduction by RNAi directed against c-myc in nude mice

To address the potential effects of RNAi on the inhibition of colon cancer cell growth *in vivo*, HT-29 cells (3.0×10^6) were injected s.c. into the mid-dorsum of 6-week old athymic nude mice. After three weeks, visible tumors had developed at the injection sites (mean tumor volume, 54.2 mm^3 ; $n = 15$). To determine the therapeutic effectiveness of c-myc siRNA, intratumoral injection treatments with siRNA, siRNAsc (used after dilution with PBS as the negative control) and 0.1 ml PBS alone (used as the blank control) were repeated every three days to a total of eight times, with five animals for each treatment. As shown in Fig. 5, c-myc siRNA markedly suppressed tumor growth as compared to siRNAsc or PBS alone ($P < 0.01$), indicating that RNAi targeting c-myc could exert a strong anti-tumor effect on xenografts *in vivo*. There was no statistically significant difference in the tumor growth of these mice as compared to those treated with either PBS or siRNAsc.

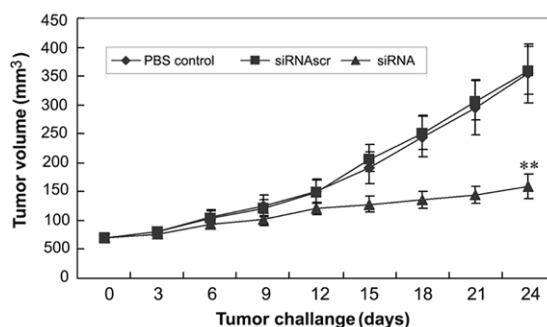


Fig. 5. The anti-tumor effect of c-myc siRNA in the HT-29 xenografts. On days 0, 3, 6, 9, 12, 15, 18, and 21, injection treatments of 0.1 ml 10 $\mu\text{mol/l}$ c-myc siRNA, siRNAsc, or 0.1 ml PBS were administered into the tumor region. Day 0 corresponds to 3 weeks after the inoculation of the cells, when the tumor volume was $50\text{--}60 \text{ mm}^3$. The tumor diameters were measured at regular intervals for up to 24 days with calipers, and the tumor volume was calculated. ** $P < 0.01$ vs. PBS alone group.

The histological analysis of the tumor xenografts by c-myc siRNA

Xenografts were processed for routine histology and immunohistochemistry using antibodies specific to c-myc. There were large areas of cytonecrosis in the c-myc siRNA-treated group compared with the siRNAsc control groups (Fig. 6A), which signified the inhibition of tumor cell proliferation. A major reduction of the level of c-myc positive cells in c-myc siRNA-treated tumors in mice was observed. The positive immunostaining was localized primarily in the cell nucleoplasm (Fig. 6B).

The inhibition of c-myc mRNA by c-myc siRNA expression in tumor xenografts

To provide evidence that siRNA anti-tumor activity against c-myc was caused by its ability to down-regulate c-myc mRNA expression *in vivo*, we analyzed treated tumor xenografts by RT-PCR analysis as previously described. Fig. 7

illustrates the mRNA of the whole tumor lysates from representative tumor xenografts. A densitometric analysis of c-myc mRNA expression, normalized to the relative GAPDH levels, demonstrated an approximately 40% reduction in the c-myc expression on day 24 in the tumors from mice treated with c-myc siRNA. By contrast, the c-myc mRNA levels detected in tumors from mice treated with siRNAscrr were equivalent to the levels of tumors treated with PBS alone. Thus, c-myc siRNA reduced the c-myc protein and mRNA levels in the tumor xenografts.

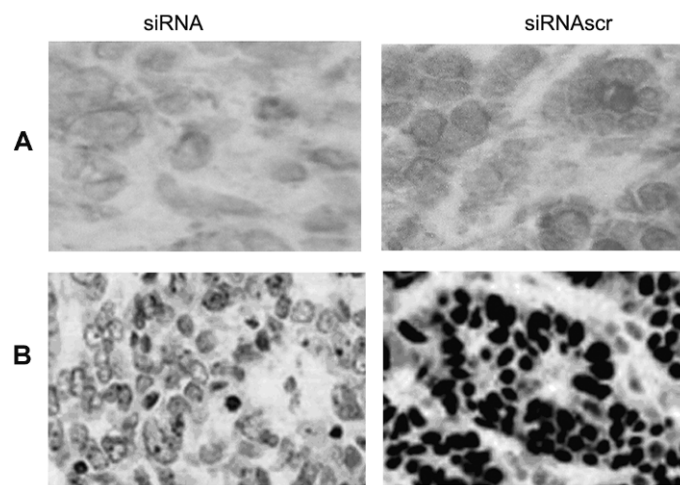


Fig. 6. Histological analyses of tumor xenografts. A – Hematoxylin and eosin (H&E) staining of tumor xenografts ($\times 400$). B – Expression of c-myc *in vivo*. Similar sections at the original magnification of $\times 200$. The siRNAscrr group (right-side microscopic fields) revealed strong expression in the cell nucleoplasm, while the siRNA group (left-side microscopic fields) showed weak expression in other areas of the cell.

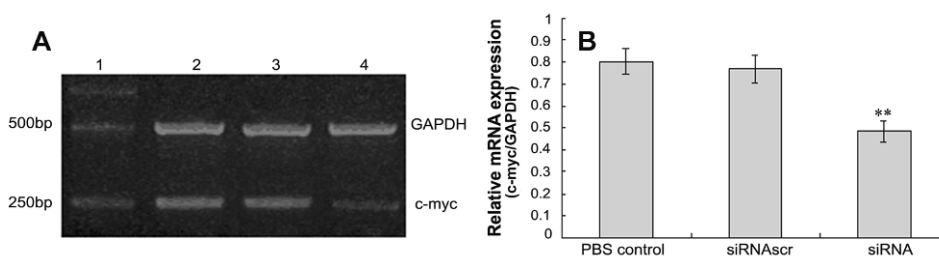


Fig. 7. The c-myc mRNA expression in excised tumors from the different groups. A – Lane 1, DNA marker; lane 2, PBS control group (blank control); lane 3, siRNAscrr-treated group; lane 4, siRNA-treated group. The mRNA levels of c-myc were detected using RT-PCR. The results shown are representative of three independent experiments. B – Quantification of the results from (A), expressed as a ratio of the expression level of c-myc GAPDH. As shown, siRNA effectively downregulated the expression of c-myc mRNA in the excised tumors (as compared to the PBS control group, $P < 0.01$).

DISCUSSION

Oncogene overexpression is frequently encountered in the development and progression of many human cancers [15]. An important oncogene, c-myc, is expressed at high levels in most human cancers, including colon, prostate, gastrointestinal, breast, lymphoma, melanoma, and myeloid leukemia [16-18]. Inactivation of this oncogene results in the sustained regression of tumors [19-21]. Only recently was this phenomenon demonstrated using mesalazine, which promotes c-myc downregulation by stimulating HT-29 cells, rendering this drug anti-proliferative and anti-carcinogenic [8]. Specific downregulation of c-myc could therefore play a role in a potential therapeutic strategy against human cancers, including colon cancer. Unfortunately, full-length antisense mRNA and oligonucleotides against c-myc mRNA [21], although previously reported to inhibit the proliferation of cancer cell lines *in vitro*, have been difficult to apply universally. Some studies even indicated that RNAi is seemingly more efficient quantitatively and more resilient in cell culture and in nude mice than antisense RNA [22].

An efficient and well-tolerated therapy for colon cancer is urgently needed, and a promising treatment approach is using RNAi with its specific downregulation of genes which promote cell proliferation, prevent apoptosis, and evade chemotherapy resistance. In this study, we demonstrated that c-myc siRNA can effectively downregulate oncogene overexpression with high specificity and suppress the cellular proliferation of colon cancer cells *in vitro* and *in vivo*.

Impaired apoptosis is also consistent with the incidence of colon cancer, and thus a more rational therapy should emerge accompanying the elucidation of processes in apoptosis-inducing agents. Concerning this, our data also suggested that knockdown of c-myc by RNAi in HT-29 cells could increase apoptosis *in vitro*. This mechanism is most probably one of the reasons behind anti-tumorigenesis. Nevertheless, there have previously been conflicting reports about the role of c-myc in apoptosis [23], i.e. c-myc is capable of both inducing and suppressing apoptosis in different types of tumor cells under different conditions and in different systems. Our data is consistent with that of Wang *et al.* [24], who determined that c-myc knockdown by RNAi in breast cancer cells could increase apoptotic sensitivity.

More studies are required to assess the pharmacokinetics and improve the tissue-specific targeting of certain genes with siRNA. Cleavage enhancement of c-myc mRNA by siRNA may also contribute to the treatment of colon cancer.

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