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Molecules and Cells



# Selection and Optimization of Asymmetric siRNA Targeting the Human c-MET Gene

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**The silencing of specific oncogenes via RNA interference (RNAi) holds great promise for the future of cancer therapy. RNAi is commonly carried out using small interfering RNA (siRNA) composed of a 19 bp duplex region with a 2-nucleotide overhang at each 3' end. This classical siRNA structure, however, can trigger non-specific effects, which has hampered the development of specific and safe RNAi therapeutics. Previously, we developed a novel siRNA structure, called asymmetric shorter-duplex siRNA (asiRNA), which did not cause the non-specific effects triggered by conventional siRNA, such as off-target gene silencing mediated by the sense strand. In this study, we first screened potent asiRNA molecules targeting the human c-MET gene, a promising anticancer target. Next, the activity of a selected asiRNA was further optimized by introducing a locked nucleic acid (LNA) to maximize the gene silencing potency. The optimized asiRNA targeted to c-MET may have potential as a specific and safe anticancer RNAi therapeutic.**

## INTRODUCTION

RNA interference (RNAi) is an evolutionarily conserved cellular process mediated by double-stranded RNA (dsRNA) and inhibits the expression of a target gene in a sequence-specific manner (Hannon, 2002). The RNAi pathway can be used by synthetic small interfering RNA (siRNA) to effectively and specifically knock-down target mRNA levels in mammalian cells (Elbashir et al., 2001a). A number of siRNA candidates has been developed and tested in clinical trials as therapies for various diseases including cancer (Davidson and McCray, 2011).

The standard siRNA structure consists of a 19 base pair duplex region with a 2-nucleotide overhang at each 3′ end, which was identified as the most efficient structure for gene silencing in early studies (Elbashir et al., 2001a; 2001b). More recent studies have determined that this siRNA structure triggers several non-specific effects, such as off-target gene silencing, saturation of RNAi machinery, and immunostimulation (Chang et al., 2011; Jackson and Linsley, 2010; Yoo et al., 2006). These non-specific effects triggered by the siRNA structure remain as hurdles to developing safe and effective RNAi therapeutics.

In our previous study, we developed a novel RNA structure that triggers RNAi, called asymmetric shorter duplex siRNA (asiRNA) (Chang et al., 2009). Compared with conventional siRNA, asiRNA has a shorter duplex region (15-16 bp) and is blunt at the 5′-end of the antisense strand. Importantly, the asiRNA structure triggered significantly fewer nonspecific effects (e.g., off-target gene silencing mediated by the sense-strand and saturation of the cellular RNAi machinery) than the conventional siRNA structure, suggesting that asiRNA could be a novel RNAi therapeutic with fewer side effects.

c-MET is a receptor tyrosine kinase (RTK) originally identified as an activated oncogene product in a human osteogenic sarcoma (HOS) cell line treated with the chemical carcinogen, N-methyl-N′-nitro-N-nitrosoguanidine (Cooper et al., 1984). Stimulation of c-MET signaling via hepatocyte growth factor/scatter factor (HGF) evokes diverse cellular responses, including survival, proliferation, angiogenesis, motility, and invasion (Ma et al., 2003). Since impaired regulation of HGF/c-Met signaling has been implicated as a factor in tumorigenesis and tumor progression, c-Met has been regarded as a potential target for cancer therapy (Christensen et al., 2005).

In the present study we aimed to develop RNAi-based anticancer therapeutics by screening and optimizing asiRNA targeted against human c-MET. After screening 30 asiRNA molecules targeted to different regions of the c-MET mRNA, the most potent asiRNA was selected. This asiRNA showed comparable gene silencing activity to a conventional siRNA, with less sense strand mediated off-target effects, consistent with the findings of our previous study. Here, we also demonstrate that the asiRNA structure can be further optimized through locked nucleic acid (LNA) modification of the sense strand.

## MATERIALS AND METHODS

### siRNA

Chemically synthesized RNA purchased from Bioneer (Korea) was used and annealed according to the manufacturer's protocol. LNA-modified RNA oligo was purchased from Exiqon (Den-

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mark). RNA sequences siMET286 were:

siMET286 antisense: 5′-CAUAUUCACAUUCAUCUCGGA-3′
siMET286 sense: 5′-CGAGAUGAAUGUGAAUAUGAA-3′

**Cell culture and siRNA transfection**

HeLa and HepG2 cells were grown and maintained in DMEM (Gibco, USA) with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were plated in 12-well plates 24 h before transfection at 30-50% confluency in complete medium without antibiotics. Transfection experiments were performed using Lipofectamine 2000 (Invitrogen, USA), G-fectin (Genolution, Korea), or Dharmafect 1 (Dharmacon, USA) at the indicated concentrations following the manufacturers' protocols.

**Quantitative RT-PCR**

A tri-reagent kit (Ambion, USA) was used to extract total RNA according to the manufacturer's protocol. The cDNA was produced from 500 ng of total RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, USA) following the manufacturer's protocol. Diluted cDNA was analyzed by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) using the StepOne™ Real-Time PCR System (Applied Biosystems, USA). Gene-specific primers were mixed with SYBR® Premix Ex Taq™ (Takara, Japan). The c-MET and GAPDH (internal control) mRNA levels were determined using the relative standard curve quantitation method. Data from a standard dilution series of the control sample were used to generate the standard curve.

The primer sequences used were:

c-MET-forward: 5′-TGGTGCAGAGGAGCAATGG-3′
c-MET-reverse: 5′-CATTCTGGATGGGTGTTTCC-3′
GAPDH-forward: 5′-GAGTCAACGGATTTGGTCGT-3′
GAPDH-reverse: 5′-GACAAGCTTCCCGTTCTCAG-3′

**Luciferase reporter assay**

DNA oligonucleotides corresponding to the sense and antisense strands of siMET286 were cloned into the *Spe*I and *Hin*dIII sites of the pMIR-REPORT Luciferase vector (Ambion, USA). DNA oligonucleotide sequences are shown below.

c-MET sense target:
5′-CTAGCATATTCACATTCATCTCG-3′
3′-GTATAAGTGTAAGTAGAGTCGA-5′
c-MET antisense target:
5′-AGCTCATATTCACATTCATCTCG-3′
3′-GTATAAGTGTAAGTAGAGCGATC-5′

A luciferase assay was performed as previously described (Chang et al., 2009).

**Western blot analysis**

Forty-eight hours after siRNA transfection, total proteins were extracted using RIPA buffer and protease inhibitors. The protein concentration was measured using a BCL protein assay kit (Pierce, USA). Equal amounts of protein were resolved via sodium dodecyl sulfate-polyacrylamide gel electrophoresis in Tris-glycine running buffer and transferred to a nitrocellulose membrane. The membrane was blocked for 1 h in Tris buffered saline buffer containing 5% skim milk powder. After overnight incubation at 4°C with a rabbit polyclonal antibody to c-MET (Santa Cruz, USA), the membrane was washed in Tris-buffered saline containing 1% Tween-20. After incubation with secondary goat anti-rabbit antibody for 1 h, the membrane was incubated with an enhanced chemiluminescence detection system (Amersham, UK) and exposed to X-ray film.

**Cell growth assay**

The growth of control and siRNA-transfected samples was measured by counting the number of cells. Cells ($2 \times 10^4$) were plated into each well of a 24-well plate, and siRNA was transfected with Lipofectamine 2000 or G-fectin following the manufacturers' protocol. Two, three, four, and five days after transfection, cells were trypsinized, diluted in the proper amount of medium, and then counted using a hemacytometer. The total cell number was calculated based on the dilution factor.

**DNA microarray**

DNA microarray experiments and analyses were performed as described previously (Hong et al., 2009). Total RNA was extracted using TRI Reagent® (Ambion, USA) and an RNeasy® mini kit (Qiagen, Germany), and 15 μg of total RNA was used to synthesize each double-stranded cDNA (dscDNA) with a commercial kit (Invitrogen). Reactions were stopped with EDTA and treated with RNase A. Samples were ethanol-precipitated and rehydrated to 250 ng/μl. One microgram of dscDNA was used for labeling with the Klenow fragment (NEB, USA) using a Cy3-labeled random 9mer (TriLink Biotechnologies, USA), and labeled samples were precipitated using isopropanol. Four micrograms of Cy3-labeled DNA (containing a sample tracking control and an alignment oligo) were hybridized to a NimbleGen 385K four-plex human microarray for 18 h at 42°C using the NimbleGen Hybridization System (NimbleGen, USA). Arrays were washed, and images were obtained using an InnoScan900 (Innopsys, France) and imported into NimbleScan software (NimbleGen, USA). Expression data was normalized through quantile normalization and the Robust Multichip Average (RMA) algorithm. For the Sylamer analysis (van Dongen et al., 2008), the 3′-UTR sequence of each human gene was retrieved from the UCSC Table Browser (Karolchik et al., 2004). The sorted gene list file and 3′-UTR sequence file were loaded into the Sylamer program, and the significantly over or under-represented k-mer sequence was computed using the Sylamer algorithm based on hypergeometric statistics under the default option, except for specified k-mer sequences to be tested.

## RESULTS AND DISCUSSION

**Selection of a potent asiRNA sequence targeting human c-MET**

In this study, we aimed to develop a candidate anticancer therapeutic using RNAi targeting the human c-MET oncogene. We used the asiRNA structure (Chang et al., 2009) to minimize the non-specific effects triggered by conventional siRNA. First, we randomly selected 30 target sequences spanning the length of the c-MET mRNA and designed a conventional siRNA and a corresponding asiRNA for each sequence (Supplementary Fig. S1). Side-by-side comparisons of gene silencing activity showed that the asiRNA was generally as efficient as the corresponding conventional siRNA at 10 nM (Fig. 1A). Out of the 30 asiRNA mole-cules, asiMET286 was the most potent c-MET gene silencer, as was the corresponding siRNA (Figs. 1A and 1B). Western blot analysis verified the reduction in c-MET protein upon asi-MET286 treatment in HeLa cells (Fig. 1C). Therefore, we used asiMET286 as an asiRNA therapeutic candidate targeting c-MET in further analyses.

**Reduced off-target silencing by asiMET286**

Several studies have demonstrated that the conventional siRNA structure can induce several non-specific effects, including off-target gene silencing (Chang et al., 2009; Sano et al., 2008; Sun et al., 2008). One cause of off-target gene silencing

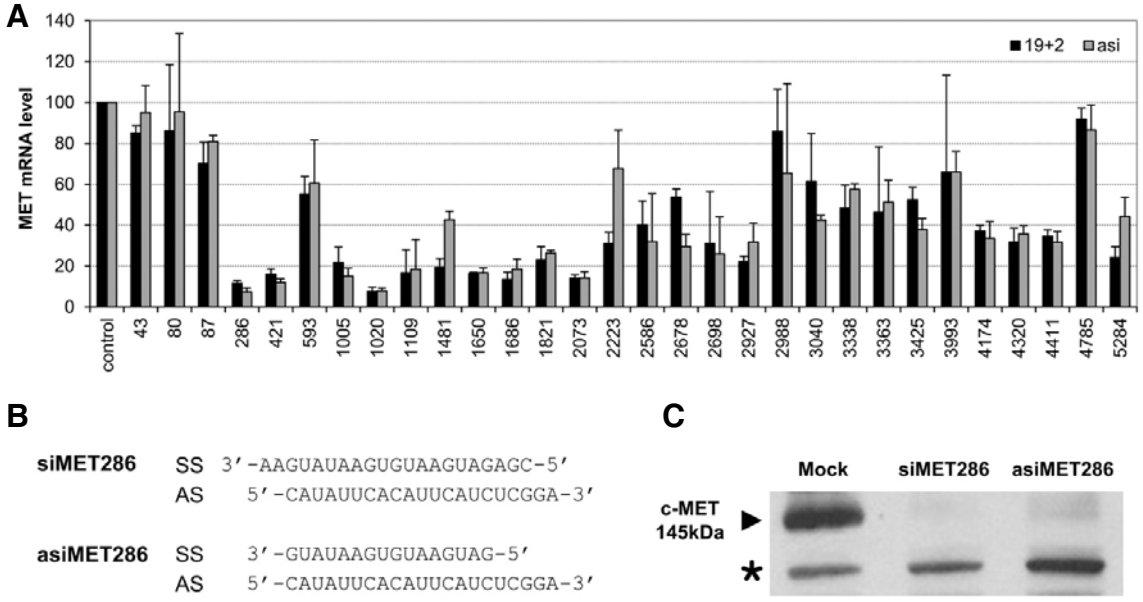


**Fig. 1.** Gene silencing activity of asiRNA targeting c-MET. (A) Reduced c-MET mRNA level induced by siRNA in HeLa cells. siRNA was transfected into HeLa cells for 24 h and c-MET mRNA levels were analyzed by quantitative real-time reverse transcription-PCR (qRT-PCR). The values plotted as "MET mRNA level" on the y-axis are normalized to GAPDH (control) mRNA level. '19+2' and 'asi' denote the conventional siRNA structure and asiRNA structure, respectively, and the control is a sham transfection. All data in the graph are mean ± SD values of three independent experiments. (B) siMET286 and asiMET286 sequences. 'SS' and 'AS' denote the sense strand and antisense strand, respectively. (C) Expression of cMET protein after siMET286 or asiMET286 treatment. HeLa cells were transfected with siMET286 or asiMET286. Cell lysates were extracted and the extracts were resolved by 8% SDS-PAGE 48 h after transfection. c-MET antibody was used to detect c-MET protein. The asterisk denotes a non-specific band used as an internal control.

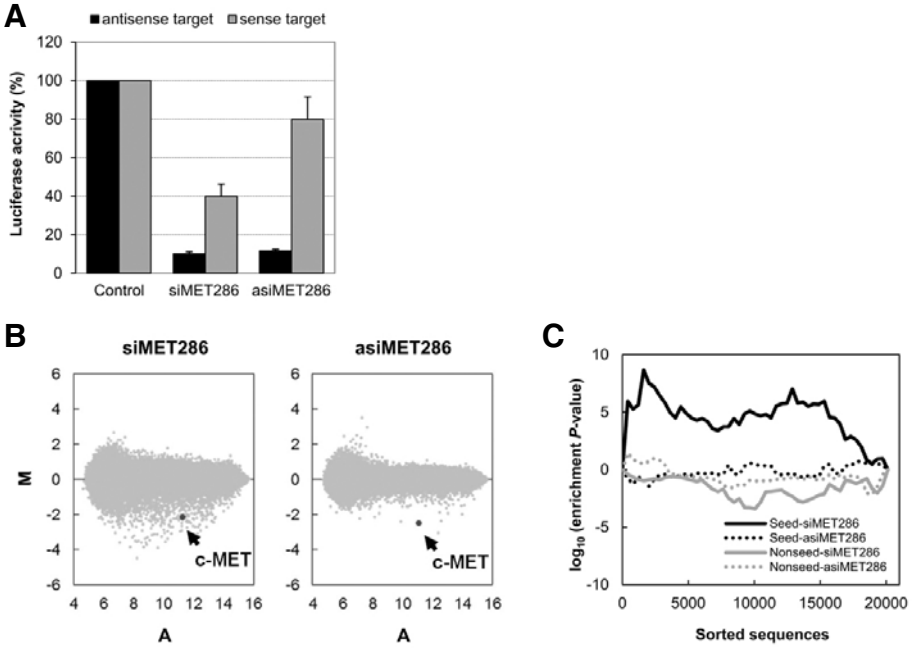


**Fig. 2.** Reduced off-target silencing by asiMET286. (A) Reduced off-target effects of asiMET286. A luciferase reporter plasmid containing the antisense or sense target sequence was transfected into HeLa cells with or without siMET286 or asiMET286. Luciferase activity was measured 48 h after transfection. The mean values + SD of three independent experiments are shown. (B, C) Validation of the reduced off-target effects of asiMET286 through DNA microarray analysis. (B) MA scatter plots illustrating genome-wide expression patterns. "M" denotes the log2-transformed expression ratios (siMET286- or asiMET286-treated versus control) and "A" denotes the average of the log2-transformed expression level. (C) Sylamer analysis for visualizing off-target effects. From the DNA microarray data, a gene list ranked according to fold change (from most down-regulated genes to most up-regulated genes) was generated and loaded into Sylamer software. Sylamer software was used to compute the enrichment of 3' UTRS targeted by the 'seed sequence' (TCATCTC) or 'non-seed sequence' (TATTCAC) of the sense strand. The solid and dotted lines represent siMET286 and asiMET286, respectively. The *x*-axes represent the sorted gene lists from most down-regulated (left) to most up-regulated (right). The *y*-axes show the hypergeometric significance of each word at each leading bin. Positive values indicate enrichment [$-\log_{10}$(*P*-value)] and negative values indicate depletion [$\log_{10}$(*P*-value)].

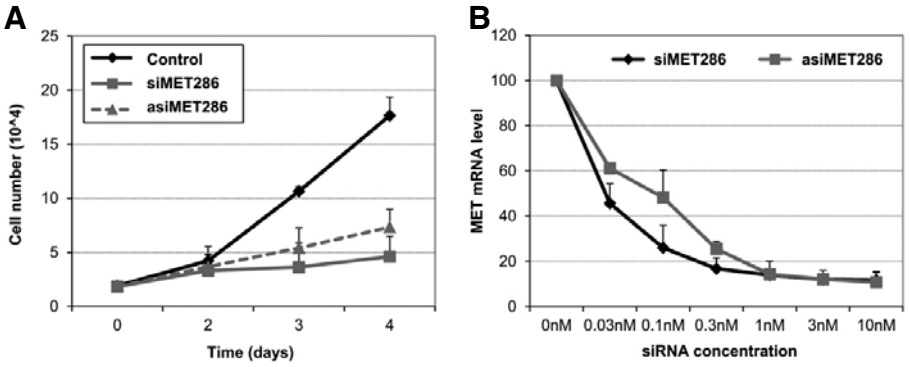


**Fig. 3.** Reduced asiRNA efficiency at low concentration. (A) Cell growth after siMET286 or asiMET286 transfection. HeLa cells were treated with each siRNA and live cells were counted at each time point (2, 3, 4 days after siRNA transfection). The control was treated with transfection reagent. Graphs represent the mean + SD of three independent experiments. (B) The $IC_{50}$ curve of siMET286 and asiMET286. HeLa cells were treated with 0.03, 0.1, 0.3, 1, 3 and 10 nM of siRNA (final concentration), and knock-down of the target mRNA was analyzed by qRT-PCR. The mean + SD of three independent experiments is shown.

is the incorporation of the sense strand of siRNA into RNA-Induced Silencing Complex (RISC), which results in silencing of non-target genes (Clark et al., 2008). We previously showed that the asiRNA structure, which has a shorter sense strand than conventional siRNA, dramatically reduced the silencing of off-target genes mediated by the sense strand (Chang et al., 2009).

To determine whether asiMET286 also reduces off-target silencing mediated by the sense strand, a luciferase reporter assay was performed with the pMIR vector containing the sense or antisense sequence of siMET286. siMET286 exhibited strong silencing activity toward the antisense target and relatively high activity toward the sense target (Fig. 2A). In contrast, asiMET286 showed strong activity toward the antisense target, but significantly less activity toward the sense target (Fig. 2A).

We performed a DNA microarray experiment to verify the reduced silencing of off-target sequences by asiMET268 at the genome level. The changes in gene expression induced by siMET286 or asiMET286 transfection are shown in an M-A plot (Fig. 2B). asiMET286 perturbed global gene expression less than siMET286, while both siMET286 and asiMET286 effectively knocked-down c-MET mRNA.

To address more specific off-target effects mediated by the sense strand, we used the Sylamer algorithm to assess over- and under-representation of nucleotide words (sequence motifs) in ranked gene lists. From the DNA microarray data, gene lists ranked according to fold change (from the most down-regulated genes to the most up-regulated genes) upon siMET286 or asiMET286 transfection were generated and loaded into the Sylamer software. The enrichment of sequences with 3′ UTRs was computed through hypergeometric statistics using the Sylamer algorithm by targeting the seed sequence of the sense strand (TCATCTC). As a control, we performed the same analysis using the non-seed sequence (TATTCAC). As shown in Fig. 2C, the Sylamer analysis of gene lists from the siMET286 array revealed that genes with the siMET286 seed sequence in the 3′UTR were down-regulated. However, the gene lists from the asiMET286 array data did not show specific region enriched pattern which means that transfection of asiMET286 did not affect expression of genes with the seed sequence in the 3′UTR. This result corroborates our theory that the asiRNA has fewer off-target effects mediated by the sense strand. In contrast, the non-seed sequence Sylamer analysis of both siMET286 and asiMET286 array data is not expected to show specific region enriched pattern and we observed common, even distribution patterns for both siMET286 and asiMET286 when the non-seed sequence was used for analysis.

**Anti-cancer activity of asiMET286**

Next, we evaluated the ability of asiMET286 to inhibit cancer cell growth. siMET286 or asiMET286 (10 nM) was transfected into HeLa cells using G-fectin, and the number of viable cells was counted two, three, and four days after transfection. While siMET286 and asiMET286 effectively and similarly reduced both mRNA and protein expression (Figs. 1A and 1C), asiMET286 showed less inhibition of cancer cell growth than siMET286 at all time points (Fig. 3A and Supplementary Fig. S2).

One explanation for the decreased inhibition of cell growth by asiMET286 is that the $IC_{50}$ value of asiMET286 (~90 pM) is higher than that of siMET286 (~28 pM), resulting in a reduced long-term gene silencing activity. Indeed, asiMET286 was less efficient at gene silencing than siMET286 at low concentrations (Fig. 3B). Thus, while the gene silencing activity of asiMET286 is comparable to that of siMET286 up to 1 nM, asiMET286 has reduced activity at lower concentrations, which results in reduced anti-cancer activity.

Off-target effects caused by siRNA transfection can induce toxic phenotypes, such as cell death (Fedorov et al., 2006). siRNA containing the UGGC sequence motif tends to evoke toxic phenotypes. AU-rich pentamers (AUUUG, GUUUU, AUUUU, CUUUU, UUUUU, and GUUUG) have also been suggested as toxic motifs. The siRNA sequences used in our study did not contain toxic sequence motifs. In addition, DNA microarray data showed no significant change in the expression of cell death- or cell cycle-related genes (Supplementary Fig. S3). Therefore, we can conclude that cell growth inhibition by siMET or asiMET is the result of RNA interference.

**Locked-nucleic acid (LNA) modification enhances the gene silencing and anti-proliferative activity of asiMET286**

We hypothesized that asiMET286 might have reduced gene silencing activity at low concentrations because of the relatively low thermal stability of the asiRNA duplex, since the duplex length is 16 bp instead of 19 bp. We used LNA modification of the sense strand of asiMET286 to enhance the molecule's thermostability. The ribose moiety of LNA is modified with an extra methylene linkage between the 2′-oxygen and the 4′-carbon of the ribose ring. This linkage provides high affinity hybridization to complementary bases (Kaur et al., 2006; Vester and Wengel, 2004). As a result, LNA modification significantly increases the melting temperature (Tm) of the duplex.

We subjected three adenines on the sense strand of asiMET286 to LNA modification (Fig. 4A) and determined whether this modification increased the gene silencing activity. As predicted, the LNA-modified asiRNA (LNA-asiMET286) had

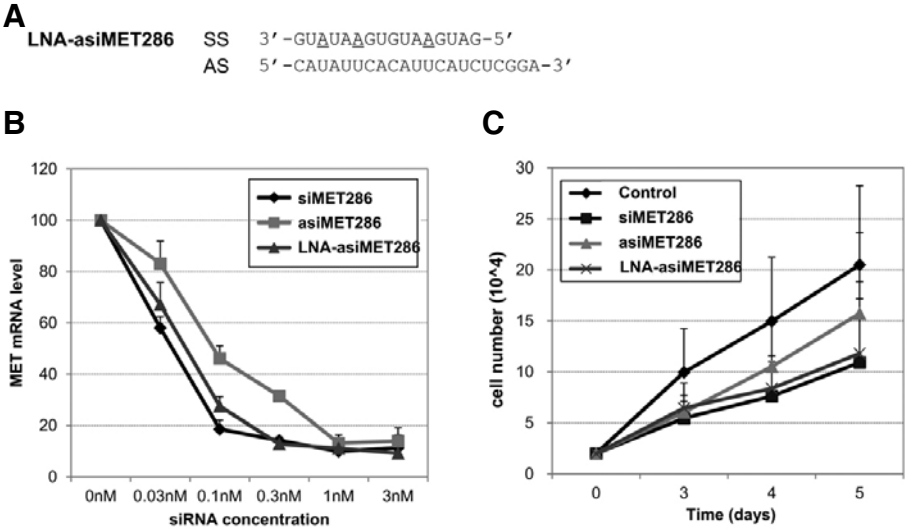


**Fig. 4.** Enhanced therapeutic effect of asiRNA by LNA modification. (A) LNA-asiMET286 sequence. LNA modification sites are underlined. (B) $IC_{50}$ curve of LNA-asiMET286. HeLa cells were treated with 0.03, 0.1, 0.3, 1, 3 and 10 nM of siRNA (final concentration) and knock-down of the target mRNA was analyzed by qRT-PCR. The mean + SD of two independent experiments is shown. (C) Cell growth upon siRNA treatment of HepG2 cells using G-fectin transfection reagent. Graphs represent the mean + SD of two independent experiments.

enhanced gene silencing activity compared with unmodified asiRNA (Fig. 4B). LNA-asiMET286 had a gene silencing efficiency nearly similar to that of siMET286 at all tested concentrations. Consistent with the improved gene silencing activity, LNA-asiMET286 showed increased inhibition of cancer cell growth, which was similar to that of siMET286 (Fig. 4C). Long-term gene silencing was also enhanced by LNA modification and was comparable to that by siMET286 (Supplementary Fig. S2). We also confirmed that LNA modification did not produce off-target effects (Supplementary Fig. S4). These results indicate that LNA modification improves the gene silencing activity of the asiRNA structure, most likely by increasing the thermostability of the asiRNA duplex.

In this study, we optimized the asiRNA structure using a chemical modification. After screening different asiRNA molecules that target c-Met, we found a potent asiRNA, asiMET286, with an $IC_{50}$ value of ~90 pM. Both a luciferase reporter assay and a DNA microarray verified that the asiRNA structure reduced off-target effects. In addition, we chemically modified the asiRNA structure. LNA-modified asiMET286 showed enhanced gene silencing activity, as well as anti-proliferative activity.

An advantage of specific and potent gene targeting makes RNAi one of the most promising disease treatment platforms. However, accumulating evidence has identified drawbacks of this technology, including off-target effects mediated by the sense strand and stimulation of the innate immune response. Recently, a phase 3 clinical trial of a first-in-class siRNA drug targeting vascular endothelial growth factor (VEGF) was terminated because the siRNA stimulated the sequence non-specific toll-like receptor 3 (TLR3) (Kleinman et al., 2008). Many groups have tested chemical or structural modifications to overcome the current hurdles of siRNA technology (Chu and Rana, 2008; Gaglione and Messere, 2010; Grimm, 2009; Sano et al., 2008; Sun et al., 2008). asiRNA, developed by our group, is one structural variation with reduced side effects (Chang et al., 2009). We expected a synergistic effect in terms of efficacy based on our inclusion of an additional chemical modification to our structure to stabilize the asiRNA duplex. The resulting data suggested remarkably increased RNAi activity. We believe that chemically modified asiRNA molecules are potent candidates for RNAi cancer therapeutics. In addition, our results suggest that substantial improvement in RNAi technology is possible by manipulating existing RNAi structures. We are currently attempting to develop a next-generation RNAi platform by chemically and structurally modifying siRNA.

*Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).*

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## REFERENCES

Chang, C.I., Yoo, J.W., Hong, S.W., Lee, S.E., Kang, H.S., Sun, X., Rogoff, H.A., Ban, C., Kim, S., Li, C.J., et al. (2009). Asymmetric shorter-duplex siRNA structures trigger efficient gene silencing with reduced nonspecific effects. Mol. Ther. *17*, 725-732.

Chang, C.I., Kim, H.A., Dua, P., Kim, S., Li, C.J., and Lee, D.K. (2011). Structural diversity repertoire of gene silencing small interfering RNAs. Nucleic Acid Ther. *21*, 125-131.

Christensen, J.G., Burrows, J., and Salgia, R. (2005). c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. Cancer Lett. *225*, 1-26.

Chu, C.Y., and Rana, T.M. (2008). Potent RNAi by short RNA triggers. RNA *14*, 1714-1719.

Clark, P.R., Pober, J.S., and Kluger, M.S. (2008). Knockdown of TNFR1 by the sense strand of an ICAM-1 siRNA: dissection of an off-target effect. Nucleic Acids Res. *36*, 1081-1097.

Cooper, C.S., Park, M., Blair, D.G., Tainsky, M.A., Huebner, K., Croce, C.M., and Vande Woude, G.F. (1984). Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature *311*, 29-33.

Davidson, B.L., and McCray, P.B., Jr. (2011). Current prospects for RNA interference-based therapies. Nat. Rev. Genet. *12*, 329-340.

Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001a). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature *411*, 494-498.

Elbashir, S.M., Martinez, J., Patkaniowska, A., Lendeckel, W., and Tuschl, T. (2001b). Functional anatomy of siRNAs for mediating efficient RNAi in Drosophila melanogaster embryo lysate. EMBO J. *20*, 6877-6888.

Fedorov, Y., Anderson, E.M., Birmingham, A., Reynolds, A., Karpilow, J., Robinson, K., Leake, D., Marshall, W.S., and Khvorova, A. (2006). Off-target effects by siRNA can induce toxic phe-

notype. RNA *12*, 1188-1196.
Gaglione, M., and Messere, A. (2010). Recent progress in chemically modified siRNAs. Mini-Rev. Med. Chem. *10*, 578-595.
Grimm, D. (2009). Small silencing RNAs: state-of-the-art. Adv. Drug Deliv. Rev. *61*, 672-703.
Hannon, G.J. (2002). RNA interference. Nature *418*, 244-251.
Hong, S.W., Yoo, J.W., Kang, H.S., Kim, S., and Lee, D.K. (2009). HIF-1alpha-dependent gene expression program during the nucleic acid-triggered antiviral innate immune responses. Mol. Cells *27*, 243-250.
Jackson, A.L., and Linsley, P.S. (2010). Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. Nat. Rev. Drug Discov. *9*, 57-67.
Karolchik, D., Hinrichs, A.S., Furey, T.S., Roskin, K.M., Sugnet, C.W., Haussler, D., and Kent, W.J. (2004). The UCSC Table Browser data retrieval tool. Nucleic Acids Res. *32*, D493-496.
Kaur, H., Arora, A., Wengel, J., and Maiti, S. (2006). Thermodynamic, counterion, and hydration effects for the incorporation of locked nucleic acid nucleotides into DNA duplexes. Biochemistry *45*, 7347-7355.
Kleinman, M.E., Yamada, K., Takeda, A., Chandrasekaran, V., Nozaki, M., Baffi, J.Z., Albuquerque, R.J., Yamasaki, S., Itaya, M., Pan, Y., et al. (2008). Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. Nature *452*, 591-597.
Ma, P.C., Maulik, G., Christensen, J., and Salgia, R. (2003). c-Met: structure, functions and potential for therapeutic inhibition. Cancer Metastasis Rev. *22*, 309-325.
Sano, M., Sierant, M., Miyagishi, M., Nakanishi, M., Takagi, Y., and Sutou, S. (2008). Effect of asymmetric terminal structures of short RNA duplexes on the RNA interference activity and strand selection. Nucleic Acids Res. *36*, 5812-5821.
Sun, X., Rogoff, H.A., and Li, C.J. (2008). Asymmetric RNA duplexes mediate RNA interference in mammalian cells. Nat. Biotechnol. *26*, 1379-1382.
van Dongen, S., Abreu-Goodger, C., and Enright, A.J. (2008). Detecting microRNA binding and siRNA off-target effects from expression data. Nat. Methods *5*, 1023-1025.
Vester, B., and Wengel, J. (2004). LNA (locked nucleic acid): high-affinity targeting of complementary RNA and DNA. Biochemistry *43*, 13233-13241.
Yoo, J.W., Hong, S.W., Kim, S., and Lee, D.K. (2006). Inflammatory cytokine induction by siRNAs is cell type- and transfection reagent-specific. Biochem. Biophys. Res. Commun. *347*, 1053-1058.