miR-221 Silencing Blocks Hepatocellular Carcinoma and Promotes Survival

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Abstract
Patients with advanced hepatocellular carcinoma (HCC) face a dismal prognosis because of a lack of any effective therapies. To address this situation, we conducted a preclinical investigation of the therapeutic efficacy of oligonucleotides directed against the oncopgenic microRNA miR-221, which has been implicated in HCC. Of 9 chemistries evaluated, we determined that a 2′-O-methyl phosphorothioate-modified anti-miR-221 oligonucleotide was most effective at reducing proliferation in vitro. A cholesterol-modified isofom of anti-miR-221 (chol-anti-miR-221) exhibited improved pharmacokinetics and liver tissue distribution compared with unmodified oligonucleotide. Chol-anti-miR-221 significantly reduced miR-221 levels in liver within a week of intravenous administration and in situ hybridization studies confirmed accumulation of the oligonucleotide in tumor cells in vivo. Within the same period, chol-anti-miR-221 reduced tumor cell proliferation and increased markers of apoptosis and cell-cycle arrest, elevating the tumor doubling time and increasing mouse survival. Taken together, our findings offer a preclinical proof of efficacy for chol-anti-miR-221 in a valid orthotopic mouse model of HCC, suggesting that this targeted agent could benefit treatment for patients with advanced HCC. Cancer Res; 71(24): 7608–16. ©2011 AACR.

Introduction
Hepatocellular carcinoma (HCC) is the third most common cause of cancer death worldwide. Accounting for an estimated 549,000 deaths per year, it represents 10% of all deaths from cancer (1). At its earliest stages, HCC is treatable by resection or transplantation. Percutaneous ablation is an option in patients who are afflicted with early HCC and who are not candidates for resection or transplantation (2). Transarterial chemoembolization has been effective in patients with intermediate stage HCC (3). Patients with HCC who are diagnosed with advanced disease or whose cancer recurs following regional therapy have a dismal prognosis. Doxorubicin chemotherapy produces response rates of 10% to 15% with no survival advantage (4). Combination therapy with cisplatin/interferon/doxorubicin/5-fluorouracil produced somewhat higher response rates than single agent doxorubicin, however, at the expense of significantly higher toxicity (5, 6). New therapies for HCC include epidermal growth factor receptor inhibitors (7) and antiangiogenic compounds such as bevacizumab (8, 9) and sunitinib (10). In a phase III trial, patients with advanced HCC treated with the molecular targeted agent sorafenib, reported an increase in survival of approximately 3 months (11). Clearly, new agents must be developed to treat advanced HCC.

Extensive profiling studies over the past several years have shown that various miRNAs are differentially expressed in HCC and other types of cancers (12–20). miRNAs with increased expression in the tumor often target tumor suppressors. Differentially expressed miRNAs in HCC include miR-21, miR-221, miR-21, and miR-18 (increased expression in HCC) and miR-122a, miR-199a*, and miR-200 (reduced expression in HCC; reviewed in refs. 21, 22). In the present study, we focused our attention on miR-221 as a therapeutic target. miR-221 expression was increased in the HCC tumors compared with non-diseased and adjacent benign liver (12–14, 18, 20). In highly aggressive HCCs, miR-221/222 was among the most upregulated of all miRNAs studied (18). miR-221 targets a number of key tumor suppressors including p27kip1 (23–25), p57kip2 (26, 27), phosphatase and tensin homolog (PTEN; ref. 28), a tissue inhibitor of metalloproteinase-3 (TIMP3; ref. 28), and the DNA damage-inducible transcript 4 (DDIT4), a modulator of the mTOR pathway (18). miR-221 antisense oligonucleotides reduced in vitro cell proliferation in HCC (18) and pancreatic cancer (29).

Antisense oligonucleotides targeted to miRNA are effective in mice (30, 31) and primates (32, 33). A human clinical trial

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using anti-miR-122 to treat hepatitis C infection has recently been completed (Santaris Pharma, ClinicalTrials.gov). An issue that has impeded the use of antisense oligonucleotides is the delivery and cellular accumulation to the target organ. It is well known that antisense and other oligonucleotides preferentially accumulate in highly perfused organs such as the liver. Therefore, targeting the liver with anti-miRNA oligonucleotides presents an ideal opportunity. Injection of 2’-MOE antisense oligonucleotides to the liver-specific miR-122 was effective at miR-122 degradation, target gene upregulation and cholesterol lowering in mice (30). Locked nucleic acid (LNA) antisense oligonucleotides to miR-122 administered to monkeys resulted in depletion of mature miR-122 and reduced plasma cholesterol levels (32). Primates dosed with LNA antisense to miR-122 had reduced hepatitis C virus viral load without evidence of viral resistance or side effects (33).

The purpose of this study was to evaluate anti-miR-221 oligonucleotides as a potential therapeutic for HCC in mice. Our goal was to show that anti-miR-221 oligonucleotides could be delivered to liver tumors and be effective in modulating miRNA expression and produce a functional effect on validated targets in orthotopic human HCC xenografts. We purposed that anti-miR-221 oligonucleotides would accumulate in the HCC tumors, reduce endogenous miR-221 oligonucleotides, modulate miR-221–related protein levels, and enhance the survival of tumor-bearing mice. Our results show the successful accomplishment of these goals.

Materials and Methods

Cell lines

The PLC/PRF/5 cell line was purchased from American Type Tissue Collection prior to 2005. The PLC/PRF/5 cells were authenticated using short tandem repeat analysis in March, 2011 by Johns Hopkins University. Our analysis showed that the cells were successfully authenticated. Cells were cultured in RPMI-1640 medium with 10% FBS using standard conditions. PLC/PRF/5 cells were stably transfected with luciferase (luc) expressing construct to generate PLC/PRF/5-luc cells.

Oligonucleotides

miR-221 antisense and scrambled control oligonucleotides were synthesized from Thermo Fisher. Oligonucleotides contained the natural nucleotides and phosphodiester bond or were chemically modified to contain a phosphorothioate (PS) linkage, 2’-OME, 2’-F, LNA, 5’ cholesterol, or a 3’ inverted deoxynucleosine (idT; Supplementary Table S1). The oligonucleotides for the mouse studies were processed for in vivo work by the manufacturer and include sterilization and testing for endotoxin.

Antisense oligonucleotide transfection

The cells (1,200 cells per well) were plated 1 day before transfection and the oligonucleotides were transfected using Lipofectamine 2000 and Opti-MEM medium (Invitrogen) following the manufacturer’s protocol. Transfection efficiency was measured using a DY547 fluorescently labeled miR-16 oligonucleotide.

Cell proliferation assay

Cell proliferation assay was carried out using the reagent WST-1 (Roche). Cells were seeded into 96-well plates at 1,200 cells per well. On the following day, the cells were transfected with anti-miRNA oligonucleotides and allowed to incubate for a predetermined time (e.g., 48 hours). Fifteen microliters of WST-1 was then added to the cell culture medium and incubated for 2 hours. Sample absorbance was analyzed using a microplate ELISA reader at 490 nm. All experiments were carried out at least in triplicate.

Quantitative PCR of microRNA

The mature miRNA was quantified by quantitative PCR (qPCR) using the TaqMan microRNA Assays (Applied Biosystems). The references gene was 18S rRNA. qPCR (10 µL reaction) was carried out using 1 µL of a 1:50 dilution of cDNA. Duplicate PCRs were carried out for each miRNA. The mean Ct was determined from the duplicate PCRs and the data were presented using the comparative Ct method.

Pilot pharmacokinetic studies

All animal experiments were carried out under protocols approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee. Female C57BL/6 mice were obtained from Harlan Sprague Dawley and maintained in a clean room with a 12-hour light–dark cycle and provided food and water ad libitum. A cholesterol-modified isoform of anti-miR-221 (cholesteryl-modified isoform of anti-miR-221) and unmodified anti-miR-221 (non-cholesteryl-modified isoform of anti-miR-221) were freshly formulated with PBS and administrated to mice through tail vein injection at the dose of 7.5 mg/kg. After administration, mice were euthanized at various time points between 5 minutes and 36 hours with carbon dioxide followed immediately by exsanguination via cardiac puncture. Blood samples were transferred to lithium heparin tubes and centrifuged at 6,000 × g for 2 minutes. Liver tissue was flash frozen. Liver and plasma were stored at −80°C until analysis.

qPCR of anti-miR-221 for pharmacokinetic analysis

To conduct the pharmacokinetic studies, we developed a novel qPCR method to assay anti-miR-221 in plasma and tissue (additional details provided in the legend to Supplementary Fig. S1). Because the 3’ cholesterol interfered with the reverse transcription (data not shown), the cholesterol label was moved to the 5’ end of the oligo and in its place a 3’ inverted deoxynucleosine (idT; Supplementary Table S1). The oligonucleotides for mouse studies were processed for in vivo work by the manufacturer and include sterilization and testing for endotoxin.

The mature miRNA was quantified by quantitative PCR (qPCR) using the TaqMan microRNA Assays (Applied Biosystems). The references gene was 18S rRNA. qPCR (10 µL reaction) was carried out using 1 µL of a 1:50 dilution of cDNA. Duplicate PCRs were carried out for each miRNA. The mean Ct was determined from the duplicate PCRs and the data were presented using the comparative Ct method.
Accuracy and precision of the PCR assay were determined for both intra- and interruns. Coefficient of variation is an indication of precision. The percentage relative error at three concentrations of oligonucleotide, representing the entire range of samples analyzed, was summarized in Supplementary Table S2.

Pharmacokinetic analysis
Pharmacokinetic data analysis and modeling were carried out using WinNonlin v.5.2 (Pharsight). Noncompartmental analyses were completed with uniform weighting and linear trapezoidal AUC calculations. For compartmental analysis, 2- and 3-compartment models were used for non-chol-anti-miR-221 and chol-anti-miR-221, respectively.

Western blot analysis
Protein was harvested using CellLytic MT (Sigma-Aldrich) and 1 x protease and phosphatase inhibitor (Pierce) using standard techniques. Protein concentration was measured using the BCA Protein Assay Kit (Pierce). Thirty micrograms of total protein extract was separated on a 10% SDS-PAGE gel. Blotting was carried out for p27Kip1, p57kip2, and PTEN (Santa Cruz Biotechnology). β-Actin (Abcam) was used as the loading control. Secondary horseradish peroxidase antibody was detected using the ECL Western Blotting Analysis System (Amersham Biosciences).

Northern blotting
Total RNA was isolated from the frozen mouse tissues using TRIzol reagent (Life Technologies) or RNeasy Plus Mini kit (Qiagen) following the manufacturer’s protocol. Northern blotting was carried out as previously described (35). LNA probes specific to the mature miR-221 were purchased from Exiqon.

Establishment of murine orthotopic HCC xenografts
Orthotopic tumors were established by the direct intrahepatic injection of PLC/PRF/5-luc cells (1,000,000 cells suspended in Matrigel) into the left hepatic lobe. Starting 10 to 14 days after orthotopic implantation, and every week thereafter, tumor burden was determined by bioluminescence imaging using the IVIS200 Imaging System (Xenogen Corp.). 10 minutes after intraperitoneal administration of 150 mg/kg body weight O-luciferin (Gold Biotechnology). Once bioluminescence exceeded 1 x 10^6 photons/s, mice were randomized to receive either 60 mg/kg chol-anti-miR-221 (n = 7) or chol-labeled scrambled control antisense oligonucleotide (n = 5) 3 days a week, for 2 weeks, intravenously. Body weight was measured and bioluminescence imaging was carried out weekly for the first 4 weeks. Tumor doubling time was calculated by modeling the data to the standard equation for exponential growth (A_t = A_0e^{kt}). Mice were followed for 10 weeks and survival curves analyzed using the Kaplan–Meier method.

In situ detection of miRNA and immunohistochemistry
LNA probes that hybridized to endogenous miR-221 and to the miR-221 antisense oligonucleotide and 5’ end-labeled with digoxigenin were purchased from Exiqon. In situ hybridization was carried out on the FFPE sections of tumor tissue as described (36) and were blinded to the experimental conditions. Nuclear fast red was used as the counterstain. p27Kip1, Ki-67, and cleaved caspase-3 (Santa Cruz Biotechnology) were detected using standard immunohistochemistry techniques. Colocalization analysis was carried out with the Nuance system from Cambridge Research Institute (Woburn, MA) according to their specifications.

Results

Evaluation of chemically modified anti-miR-221
The natural oligonucleotide chemistry (2’-OH and phosphodiester backbone) as well as 8 chemical modifications of the anti-miR-221 and scrambled control oligonucleotides (Supplementary Table S1) were evaluated. The anti-proliferative activity of the modified anti-miR-221 or scrambled control oligos was studied in PLC/PRF/5 cells following Lipofectamine transfection. The most effective chemical modification at reducing cell proliferation was the 2’-OMe. Ps; reducing cell proliferation by 25% at both the 48- and 72-hour time points (Supplementary...
Antisense Inhibition of MicroRNA in Hepatocellular Carcinoma

Pharmacokinetics of cholesterol and non–cholesterol-labeled anti-miR-221

Pilot studies were completed to evaluate plasma pharmacokinetics and liver distribution of both chol-anti-miR-221 and non-chol-anti-miR-221 in C57BL/6 mice. Figure 1 displays concentration–time profiles and model fits for the plasma and liver data. Estimated noncompartmental pharmacokinetic parameters are provided in Table 1. A comparison of the chol-anti-miR-221 and non-chol-anti-miR-221 data indicates the cholesterol modification significantly alters anti-miR-221 oligonucleotide pharmacokinetics. Figure 1A indicates a prolonged distribution phase of chol-anti-miR-221, resulting in significantly increased plasma area under the curve (AUClast) 74.4 vs. 7.3 h µg/ml for chol-anti-miR-221 and non-chol-anti-miR-221, respectively). This altered pharmacokinetic behavior is reflected in the liver data (Fig. 1B), which indicates approximately 50-fold higher maximum concentrations (Cmax) and AUCs of chol-anti-miR-221 than non-chol-anti-miR-221. The modified pharmacokinetic properties of chol-anti-miR-221 show the cholesterol modification achieves the desired effects of increasing concentrations and prolonging exposures of the anti-miR-221 in liver tissue.

Hepatic accumulation and activity of chol-anti-miR-221 in normal and tumor-bearing mice

C57BL/6 mice were injected via the tail vein with 7.5 mg/kg of chol-anti-miR-221 3 times over a 10-day period; mice were then sacrificed at days 11, 14, and 17. The amount of endogenous miR-221 in the whole liver was reduced by about 2-fold at day 11, but the effect was short lived with no significant change in miR-221 levels at days 14 and 17 (data not shown). For this reason we increased the dose to 30 mg/kg, C57BL/6 mice injected with 3 daily doses of 30 mg/kg via the tail vein were sacrificed on days 4 and 7 and the amount of chol-anti-miR-221 and endogenous miR-221 was assayed in whole liver. Both qPCR and Northern blotting showed that chol-anti-miR-221 significantly reduced the levels of endogenous miR-221 (Fig. 2A and B). The reduction in endogenous miR-221 correlated with increased accumulation of the chol-anti-miR-221 in the liver (Fig. 2A and C). Endogenous miR-221 was reduced by 80% and 90% at days 4 and 7, respectively (Fig. 2A). The expression of 4 control miRNAs was not altered by the chol-anti-miR-221 treatment (Supplementary Fig. S3).

Mice bearing xenograft orthotopic tumors were injected via the tail vein with 30 or 60 mg/kg of the chol-anti-miR-221 or cholesterol-labeled scrambled control oligo for 3 consecutive daily doses. Mice were sacrificed at days 4 and 7 and the amount of endogenous miR-221 and chol-anti-miR-221 was assayed in the tumor tissue. Similar to the effect in normal liver, chol-anti-miR-221 produced a significant reduction in the endogenous miR-221 levels in the tumor (Fig. 3A and B). The miR-221 levels in the cholesterol-labeled scrambled control oligo treatment did not differ from that of the untreated control (Fig. 3A and B). There was a trend between the accumulation of chol-anti-miR-221 (30 mg/kg dose) and endogenous miR-221 levels in the tumor between days 4 and 7; comparing the 30 and 60 mg/kg doses (day 7), endogenous

| Table 1. Noncompartment pharmacokinetic parameters for non-chol-anti-miR-221 and chol-anti-miR-221 |
|-------------------------------------------------|------------------|------------------|
| Mouse plasma                                    | Non-chol-     | Chol-anti-    |
|                                                | anti-miR-221  | miR-221        |
| T1/2, h                                        | 15.8           | 4.1            |
| AUClast, h mg/mL                               | 7.3            | 74.4           |
| Cl, mL/h/kg                                    | 1.013          | 101            |
| Mouse liver                                    |                |                |
| T1/2, h                                        | 1.5            | 6.0            |
| Cmax, ng/mL                                    | 123            | 5,654          |
| AUClast, h mg/mL                               | 2.3            | 118            |

Abbreviations: AUClast, area under the concentration–time curve from time zero to last time with the quantifiable concentration; Cl, clearance; Cmax, maximum observed concentration of an oligo after intravenous administration; T1/2, half-life.
miR-221 decreased with increasing accumulation of chol-anti-miR-221 (Fig. 3C). To determine if the chol-anti-miR-221 modulated 3 miR-221 target proteins, we assayed p27Kip1, p57Kip2, and PTEN protein levels by Western blotting. Following 7 days of treatment, the 60 mg dose of chol-anti-miR-221 increased the p27Kip1, p57Kip2, and PTEN protein levels by 3-fold (Fig. 3D).

To determine if the chol-anti-miR-221 modulated 3 miR-221 target proteins, we assayed p27Kip1, p57Kip2, and PTEN levels by Western blotting. Following 7 days of treatment, the 60 mg dose of chol-anti-miR-221 increased the p27Kip1, p57Kip2, and PTEN protein levels by 3-fold (Fig. 3D). There was a slight increase in the p27Kip1 and p57Kip2 protein in the cholesterol-labeled scrambled control oligo–treated tumors (60 mg/kg) compared with the untreated control (Fig. 3D). To determine the degree of cycling cells, we carried out Ki-67 staining on these tissues. Ki-67 staining was significantly reduced in the tumors of the mice treated with chol-anti-miR-221 than those mice treated with cholesterol-labeled scrambled control (Fig. 4A and B; Supplementary Fig. S4).

To visualize the hepatic accumulation and activity of chol-anti-miR-221, in situ hybridization was carried out in the liver and tumors of orthotopic mice treated with both chol-anti-miR-221 and cholesterol-labeled scrambled control oligo. Significant accumulation of anti-miR-221 occurred in the tumors (Fig. 4C) of mice 7 days after the initiation of therapy. The chol-anti-miR-221 was predominately localized to the cytoplasm in the tumor (Fig. 4C). Probing the tumors for endogenous miR-221 showed a complete lack of miR-221 expression in the mice treated with chol-anti-miR-221 (Fig. 4D) but not cholesterol-labeled scrambled control oligo (Fig. 4D, insert). There is no evidence of hepatocellular toxicity in the chol-anti-miR-221, cholesterol-labeled scrambled oligo (Fig. 4E and F), or saline control-treated mice (not shown). Colocalization studies were carried out to determine the spatial expression of both chol-anti-miR-221 and p27Kip1 protein. As described above,
chol-anti-miR-221 was abundant in the tumors of the treated mice (Supplementary Fig. S5A) as was expression of the p27Kip1 protein in areas that contain high levels of chol-anti-miR-221. There was a dose-dependent increase in the active, cleaved form of caspase-3 in the chol-anti-miR-221–treated tumors compared with cholesterol-labeled control oligo (Fig. 5; Supplementary Fig. S6).

**Anti-miR-221 treatment in orthotopic tumor xenograft mice**

To further study the efficacy of chol-anti-miR-221, survival studies were initiated. Nude mice implanted with PLC/PRF/5-mice (Supplementary Fig. S5A) as was expression of the p27Kip1 protein was abundant in the tumors of the treated mice (Supplementary Fig. S5A) as was expression of the p27Kip1 protein in areas that contain high levels of chol-anti-miR-221. There was a dose-dependent increase in the active, cleaved form of caspase-3 in the chol-anti-miR-221–treated tumors compared with cholesterol-labeled control oligo (Fig. 5; Supplementary Fig. S6).

**Discussion**

In these studies, we showed the feasibility and therapeutic efficacy of selectively targeting a miRNA that is overexpressed in HCC using antisense oligonucleotides. These observations are novel for several reasons. First, a survival benefit was observed using intravenous administration of a therapeutic agent in an orthotopic model of HCC. Useful preclinical information is provided using a systemic therapy to directly target intrahepatic tumors, an approach which is highly germane and relevant to human HCC. Next, antisense oligonucleotides were used to selectively target miRNA. Their use for targeting miRNA has distinct advantages compared with their use for targeting mRNA. Finally, these studies show the therapeutic efficacy of targeting a single aberrantly overexpressed miRNA, which may have multiple cancer-relevant targets.

Thus, they provide proof-of-principle for further development of miRNA targeted therapies.

Treatment of tumor-bearing mice with chol-anti-miR-221 resulted in a survival advantage compared with mice exposed to cholesterol-modified control oligonucleotides. These observations support an anti-tumoral effect of targeting miR-221, one of the most consistently overexpressed miRNA in HCC. Our observations show an increase in tumoral expression of p27Kip1, p57Kip2, and PTEN by chol-anti-miR-221. These 3 cell-cycle regulatory factors/tumor suppressors have been implicated in human HCC. Thus, alterations in these factors support reduced tumor cell-cycle progression as a mechanism by which chol-anti-miR-221 enhances survival of tumor-bearing mice. Further support for this is provided by the significant reduction of Ki-67, increase in tumor doubling time and increased cleaved caspase-3 in tumors treated with chol-anti-miR-221. Given that a single miRNA may have multiple functional targets, it is possible that other tumoral or systemic effects of chol-anti-miR-221 could also contribute to the improved survival. Further studies to elucidate and identify the mechanisms of increased survival with chol-anti-miR-221 are clearly warranted, for example, the effect of chol-anti-miR-221 on the formation of micrometastasis.

The feasibility of targeting miRNA to modulate metabolism in the liver (30), or to achieve antiviral effects (37) has been reported. Antitumor effects as a result of miRNA modulation have been shown with the use of adenoviral vectors to overexpress miRNA such as miR-26 (38). Recently, it was shown that antisense to miR-191 could reduce tumor weight in an orthotopic mouse model of HCC (39). In contrast to these studies, our study is the first to our knowledge that shows a survival advantage by directly targeting intrahepatic tumors with anti-miRNAs. Our study provides useful preclinical information to guide further development of therapeutic strategies using similar approaches in humans. We show here that systemically administered naked oligonucleotides accumulate in the normal liver and within liver tumors at very high levels. Chol-anti-miR-221 was effective at reducing endogenous miR-221 and altering miR-221 target proteins. Notably, chol-anti-miR-221 was detectable (110–160 nmol/L) in the tumors of all 3 mice treated with chol-anti-miR-221 that survived for 10 weeks (Fig. 6D). Our data parallel that of Krutzfeldt and colleagues, who showed anti-miRNA regulation from a single dose of anti-miRNA oligo 28 weeks following injection (31). The doses used...
here (i.e., 30 and 60 mg/kg) were similar to those used in other in vivo studies for targeting liver (31). It may be possible to reduce the dose by formulating the antisense oligos into nanoparticles (40). Because miR-221 is increased in a number of solid tumors including pancreas (41), non–small cell lung cancer (28), glioblastoma (42), and thyroid cancer (25), optimizing the delivery of antisense oligos may be a useful approach to target tumors besides HCC. While the 2′-O-methyl modification used here dramatically reduced nonspecific effects (43), the phosphorothioate backbone used in our study has been shown to have excellent antisense activity but with sequence-independent effects on cellular function (44, 45). For example, interaction of phosphorothioate oligos to cellular proteins such as basic fibroblastic growth factor results in nonspecific effects (45). The slight increase in p27Kip1, p57Kip2, and cleaved caspase-3 (Fig. 3D; Supplementary Fig. S6) in cholesterol-labeled scrambled control-treated tumors might be because of sequence independent activity of the oligo backbone. Lowering the amount of oligo is one possible way to minimize the nonspecific oligo effects (44).

The choice of antisense oligonucleotides to miRNA as a targeting strategy for liver cancer is attractive for several reasons. Over the past 2 decades, major efforts have been directed to develop antisense as therapeutic agents by targeting mRNA, but with disappointing results. A drawback of antisense has been inappropriate targeting and poor cellular uptake in the target tissue. Our results corroborate what has been known for many years; oligonucleotides accumulate in highly perfused tissues such as the liver. Liver uptake is enhanced by the presence of the cholesterol label. Unlike with the design of antisense for mRNA, where the optimal antisense is determined by screening dozens of potential oligos, antisense to mature miRNA is simply the reverse compliment of the mature miRNA sequence. While siRNA has many useful applications for gene knockdown, targeting miRNA with siRNA is not practical. The cytoplasmic RISC complex that cleaves the RNA target of siRNA would not be expected to cleave the nuclear pri-miRNA. Targeting the loop region of pre-miRNAs required much higher doses of siRNA than what is required for siRNA inhibition of mRNA (46). miRNA sponges represents another option to inhibit mature miRNA levels (47), however, these are vector-driven approaches. A more clinically relevant scenario to interfere with the function of the mature miRNA is to simply inhibit the mature miRNA with antisense oligos.

Deregulation of miRNAs is consistently observed in human cancers. Therefore, targeting aberrantly expressed miRNA is...
logical and attractive if critical targetable miRNA can be identified. The precise target gene effects of these interventions are not readily elucidated because of the promiscuity of potential miRNA targets. However, this does not preclude modulation of miRNA as a therapeutic strategy when identifiable effects on tumor growth can be shown. Thus, the choice of target miRNA is crucial, and careful validation is needed. While our studies validate miR-221 as a suitable target, future studies to determine the relative efficacy of targeting miR-221 compared with other miRNAs that are aberrantly expressed in HCC may be needed to identify the most optimal target miRNA for further translation into clinical trials.

References


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