

Forum Minireview

MicroRNAs and Their Therapeutic Potential for Human Diseases: MicroRNAs, miR-143 and -145, Function as Anti-oncomirs and the Application of Chemically Modified miR-143 as an Anti-cancer Drug

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Abstract. We examined the expression levels of microRNAs (miRNAs; miRs) in colorectal tumors (63 cancer specimens and 65 adenoma specimens) compared to adjacent non-tumorous tissues. Decreased expression of miR-143 and -145 was frequently observed in the adenoma and cancer samples. As the down-regulation of miR-143 and -145 was observed even in the early phase of adenoma formation, their decreased expression would appear to contribute mainly to the initiation of tumorigenesis. For clinical application, we added aromatic benzene-pyridine (BP-type) analogs to the 3'-overhang region of the RNA-strand and changed the sequences of the passenger strand in the miR-143 duplex (miR-143BPs), leading to greater activity and increased resistance to nuclease. The cell growth inhibitory effect of the chemically modified miR-143BPx *in vitro* was greater than that of the endogenous miR-143. The modified miR-143BPx showed a significant tumor-suppressive effect on xenografted tumors of human colorectal cancer DLD-1 cells. These findings suggest that miR-143 and -145 are important onco-related genes for the initiation of colorectal tumor development and that chemically modified miR-143BPx may be a candidate for an RNA medicine for the treatment of colorectal tumors.

Keywords: colorectal tumor, miR-143 and miR-145, chemically modified microRNA, xenografted tumor, tumor suppression

1. Introduction

MicroRNAs (miRNAs; miRs) are a class of non-coding RNA ranging from 20 – 23 nucleotides in length that are post-transcriptional regulators of gene expression. Recent studies indicate that miRNAs have essential roles in many basic biological processes including development, cell proliferation, differentiation, and apoptosis (1). A potential role for miRNAs in human cancer has been supported by the observation that more than 50% of miRNA genes are located at chromosomal regions, such as fragile sites, and in regions of deletion or amplification that are genetically altered in a variety of human cancers,

suggesting possible relevance of miRNAs in human cancers (2). Much evidence indicates that miRNAs are involved in human carcinogenesis as novel tumor suppressors or oncogenes (3 – 6). Previously, we demonstrated that the expression of miR-143 and -145 was severely down-regulated in the majority of human cancer cell lines (7) and particularly in colon cancers (8) and gastric cancers (9). Moreover, the growth of human colon or gastric cancer cells expressing miR-143 and -145 at low levels was significantly inhibited by their ectopic expression (7 – 11). These findings indicate the anti-oncogenic role of miR-143 and -145.

On the basis of such findings, the relationship between the expression of miR-143 and -145 and the clinico-pathological findings in human colorectal tumors is important. In contrast, RNA interference (RNAi) is now commonly used to suppress gene expression *in vitro* and

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in vivo using synthetic small interfering RNAs (siRNAs) in animals. Recent reports have demonstrated the potential use of synthetic siRNAs as therapeutic agents for cancer (12). However, for effective clinical application, degradation of synthetic siRNA by nucleases must be resolved, and a more efficient method of modifying RNA molecules and their system of delivery must be devised.

Our recent work has revealed that changing the structure of the passenger strand of the miR-143 duplex enhanced the growth inhibition of human colon cancer DLD-1 cells and that chemical modification of the 3'-overhang of miR-143 using aromatic moieties, such as benzene(B)-pyridine(P) analogs (BP-type), improved its resistance against nucleases (13, 14). The administration of such chemically modified miR-143BPs via intravenous injection resulted in a potent tumor-suppressive effect on xenografted human colon cancer cells. In the current review, we discuss the importance of miR-143 and -145 in colorectal tumors and the anti-cancer activity of chemically modified miRNAs.

2. Generation of miRNAs

Mature miRNAs are structurally similar to siRNAs produced from exogenous double-stranded RNA (dsRNA), but before reaching maturity, miRNAs must first undergo extensive post-transcriptional modifications. miRNAs are expressed from a much longer RNA-non-coding gene as a primary transcript known as pri-miRNAs that are processed in the cell nucleus by the

microprocessor complex, which consists of Drosha (an RNase III enzyme), to form a 70-nucleotide stem-loop structure called pre-miRNAs. The dsRNA portion of pre-miRNA is bound and cleaved by Dicer to produce the mature miRNA molecule. The miRNA can then be integrated into the RNA-induced silencing complex (RISC); thus, miRNA and siRNA share the same cellular machinery downstream of their initial processing. Although the siRNA-RISC complex cleaves the corresponding mRNA, the complex mainly inhibits translation of the corresponding mRNA (Fig. 1).

Figure 2 shows the 3'-overhang region (3'-dangling end) of the miRNA guide strand that is recognized by the

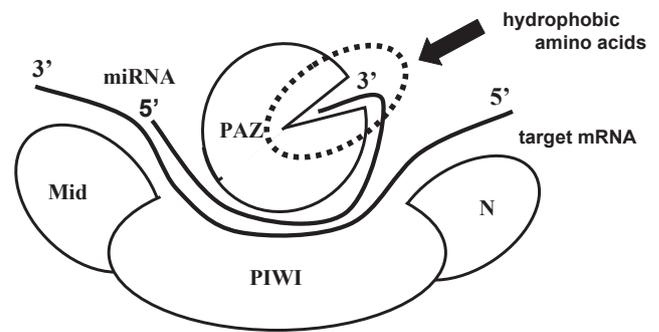


Fig. 2. Active site of the PAZ domain. The 3'-overhang region (3'-dangling end) of the miRNA guide strand that is recognized by the PAZ (Piwi/Argonaute/Zwille) domain of Ago2; 2-nucleotides of the 3'-overhang are accommodated into the binding pocket composed of hydrophobic amino acids in the PAZ domain.

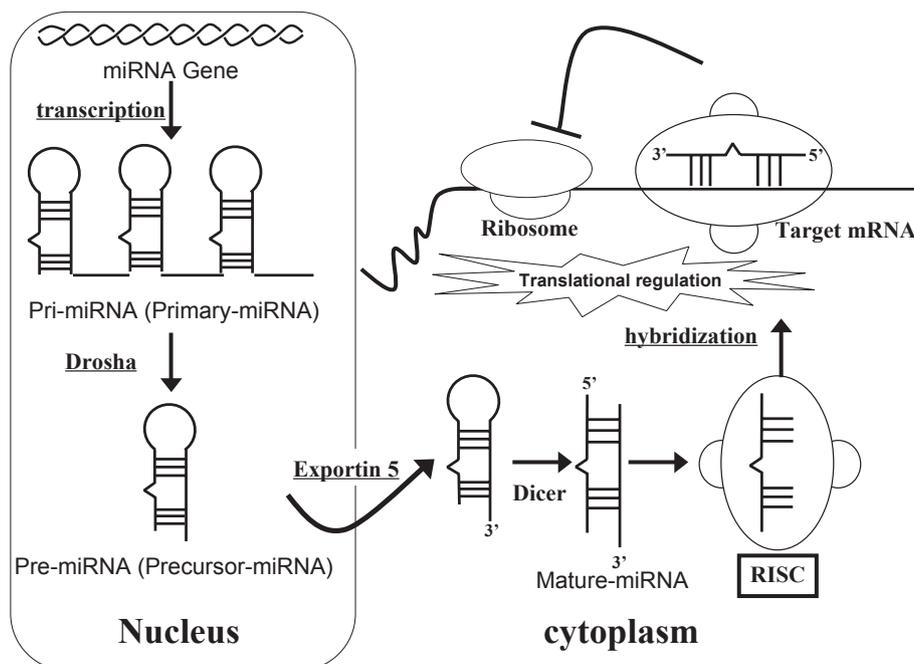


Fig. 1. Mechanism of microRNAs. miRNAs are expressed from the sequence of much longer RNA-non-coding genes. The initial step transcribes a primary transcript known as a pri-miRNA. This is processed in the cell nucleus by the microprocessor complex, which contains the RNase Drosha, to form a 70-nucleotide stem-loop structure called a pre-miRNA. The dsRNA portion of the pre-miRNA is bound and cleaved by Dicer to produce the mature-miRNA molecule. Then, the mature-miRNA can be integrated into the RISC complex, which primarily inhibits translation of the corresponding mRNA.

PAZ (Piwi/Argonaute/Zwille) domain of Ago2; 2-nucleotides of the 3'-overhang are accommodated into the binding pocket, which is composed of hydrophobic amino acids in the PAZ domain (15 – 19).

3. Biological activities of miR-143 and -145

Accumulating evidence suggests that miRNAs are involved in human carcinogenesis as novel tumor suppressors or oncogenes, such as Ras and, Bcl2 (3 – 6). Many miRNAs target mRNAs involved in processes aberrant in tumorigenesis such as cell proliferation, survival, death, and differentiation. Of all the miRNAs, miR-143 and -145 have demonstrated negative regulation of cell proliferation. This was made evident by observing their reduced expression in the majority of human cancer cell lines, particularly in colon cancers and gastric cancers (Fig. 3). We examined the expression levels of miRNAs in colorectal tumors (63 cancer specimens and 65 adenoma specimens) compared to the adjacent non-tumorous tissues (20). As down-regulation of miR-143 and -145 was observed even in the early phase of adenoma formation, the decreased expression of both miRs appears to contribute mainly to the initiation of tumorigenesis, but not to the progression stage or clinical prognostic factors. The ectopic expression of miR-143 in colon cancer DLD-1 cells down-regulated the expression of Erk5 at the translational level, as previously reported in adipocytes by Esau et al. (21). Erk5 of the MAPK superfamily plays a role of transcriptional activation of *c-myc* that results in cell growth suppression of cells (7, 9). Indeed, the silencing of Erk5 by the siRNA induced a significant cell growth inhibition (7, 8, 10). Thus, it has been accepted that miR-143 and -145 function as anti-oncomirs in colorectal cancers.

4. Chemically modified microRNAs

We found that miR-143 and -145 are down-regulated and act as anti-oncomirs in gastrointestinal tumors (7 – 9), which raises the possibility of using analogs of miR-143 and -145 as anti-cancer drugs. We previously observed that the addition of an aromatic compound to the 3'-overhang region of the RNA-strand enhances its resistance against nucleases (Fig. 4) (13). The stability of miR-143s was evaluated by measuring its level using the TaqMan miRNA assay after inoculating each miRNA into culture medium containing 5% fetal calf serum. We examined the degradation rates of 3'-chemically modified miR-143BP (3'-benzene-pyridine portion; BP type) (Fig. 5) and commercially available miR-143 (Applied Biosystems, Foster City, CA, USA) in the medium. The

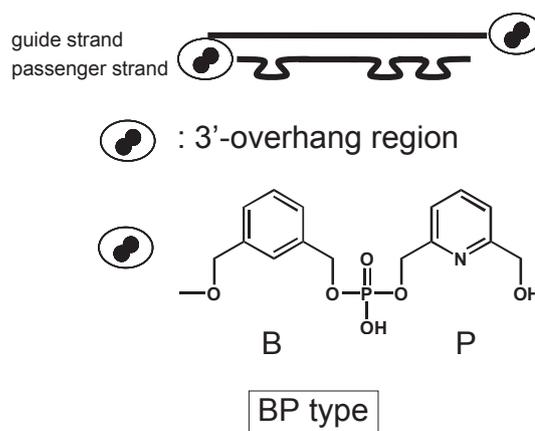


Fig. 4. Design of chemically modified miRNA. The addition of an aromatic compound such as the benzene(B)-pyridine(P) analog to the 3'-overhang region of the RNA-strand enhances its resistance against nucleases.

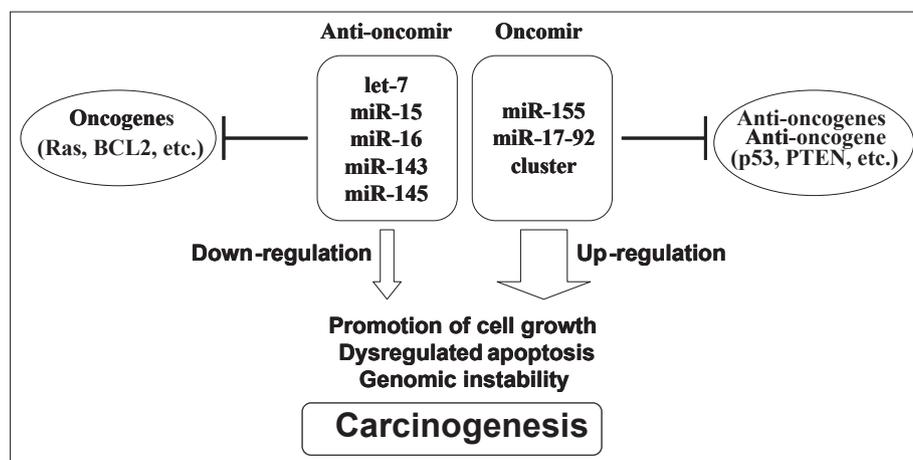


Fig. 3. Relationship between onco-related miRNAs and protein-coding oncogenes, and anti-oncogenes. An miRNA located in a deleted region, or down-regulated in a particular cancer (a tumor suppressor miRNA), can have oncogene-like effects if its main targets in cancer cells are protein-coding oncogenes. An miRNA located in an amplified region or up-regulated in a particular cancer (an oncogene miRNA) can have tumor suppressor-like effects if its main targets are protein-coding anti-oncogenes.

commercially available miR-143 was almost completely degraded within 5 min. On the other hand, more than 97% of the chemically modified miR-143 was degraded after 5 min, but thereafter, no further degradation of miR-143 occurred. The addition of BP to the 3'-overhang region of miR-143 (miR-143BP) improved its nuclease-resistance, with five- to eight-fold more miR-143BP detected in the medium when compared to miR-143 from Applied Biosystems.

We considered that the hydrophobic BP-portion at the 3'-overhang region may associate with the PAZ domain of Ago2 (Fig. 2) (13, 14). Therefore, we synthesized a

3'-modified miR-143 possessing a hydrophobic group (BP analog) at its 3'-overhang region. This 3'-modified oligonucleotide enabled a significant improvement in nuclease-resistance (Figs. 4 and 5).

5. Inhibition of cell growth by miR-143BP and -145BP in vitro

For clinical application, we further changed the passenger sequences of the mismatch portions between the passenger and guide strands. Inhibition of cell growth by the modified miR-143BP in vitro was greater than that

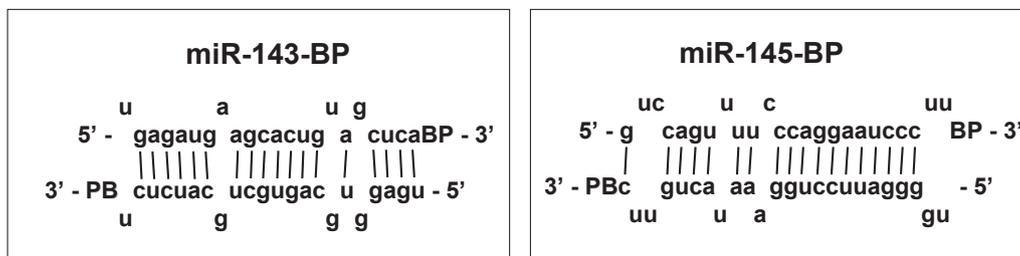


Fig. 5. Chemical modification of miR-143 and -145. mi-RNA-143BP and miR-145BP have a chemically modified benzene(B)-pyridine(P)-analog at the 3'-overhang region of both the guide strand and passenger strand. The hydrophobic BP-analog is attached to a conservative guide or passenger strand, which is the corresponding mature sequence without a 3'-overhang region.

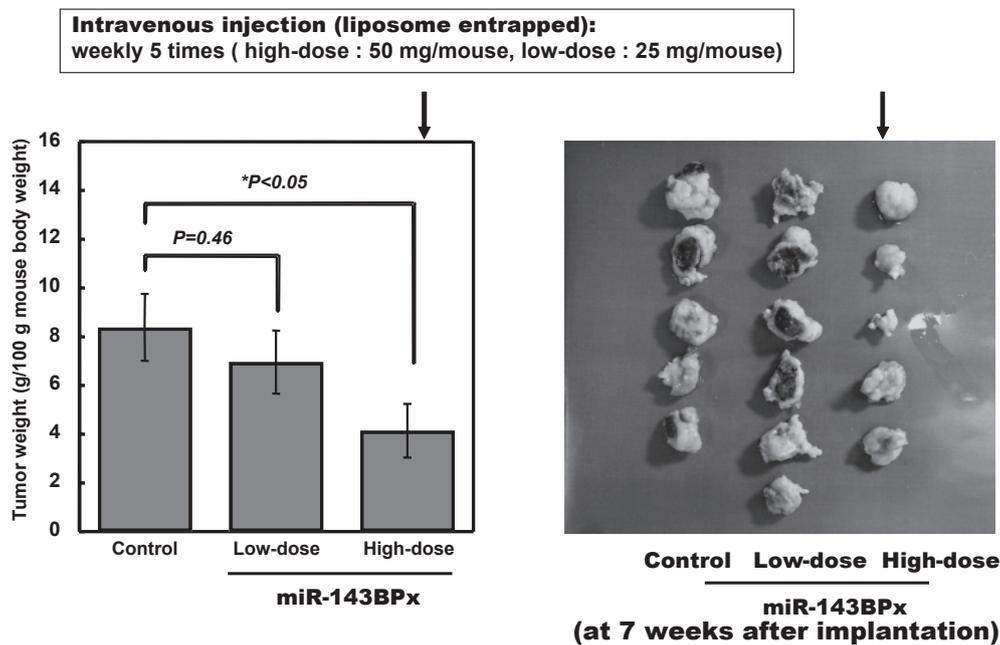


Fig. 6. Tumor-suppressive effect of chemically modified synthetic miR-143BPx in vivo. Human colon cancer DLD-1 tumors were established in athymic nude mice. When the tumors had reached approximately 300 mm³, intravenous injection treatments were started. The groups treated with oligonucleotides from Renilla luciferase served as controls. The mice were randomly assigned to one of three treatment groups. Weights of the mice were similar in all groups. *Significant difference between the bracketed groups (* $P < 0.05$).

by endogenous miR-143. Among the various modified miR-143BPs, the miR-143BPx with the mismatch sequence that was changed to match the 3'-position of the passenger strand demonstrated the most potent effects on growth inhibition (20).

6. Anticancer activities of miR-143BPx in vivo

A significant reduction in tumor size by miR-143BPx was observed from 3 weeks after weekly intravenous injections in mice xenografted with human colon cancer tumors in comparison with the control animals. At two weeks after the last injection (treatment for 5 weeks), we evaluated the tumor-suppressive effect of miR-143BP. The tumor/body weight ratios of the miR-143BPx-treated mice were dose-dependently decreased compared with those for the control miRNA (non-specific sequence) (Fig. 6) (20).

7. Summary

The 3'-modified oligonucleotides showed prominent nuclease resistance and strong RNAi activity. The preparation of modified oligonucleotides, such as miR-143BP and -145BP, for genome therapy is easy, inexpensive, and practical. Chemical modification of the 3'-dangling end of miRNAs may prove useful for the development of anti-cancer RNA medicine.

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