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Targeting microRNAs involved in human diseases: A novel approach for modification of gene expression and drug development

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ABSTRACT

The identification of all epigenetic modifications (i.e. DNA methylation, histone modifications and expression of noncoding RNAs such as microRNAs) involved in gene regulation is one of the major steps forward for understanding human biology in both normal and pathological conditions and for development of novel drugs. In this context, microRNAs play a pivotal role. This review article focuses on the involvement of microRNAs in the regulation of gene expression, on the possible role of microRNAs in the onset and development of human pathologies, and on the pharmacological alteration of the biological activity of microRNAs. RNA and DNA analogs, which can selectively target microRNAs using Watson–Crick base pairing schemes, provide a rational and efficient way to modulate gene expression. These compounds, termed antago-miR or anti-miR have been described in many examples in the recent literature and have proved to be able to perform regulatory as well as therapeutic functions. Among these, a still not fully exploited class is that of peptide nucleic acids (PNAs), promising tools for the inhibition of miRNA activity, with important applications in gene therapy and in drug development. PNAs targeting miR-122, miR-155 and miR-210 have already been developed and their biological effects studied both *in vitro* and *in vivo*.

1. Introduction

The identification of all epigenetic modifications involved in gene expression is one of the major steps forward for understanding human biology in both normal and pathological conditions. This field is referred to as epigenomics, and it is defined as epigenetic changes (i.e. DNA methylation, histone modification and expression of noncoding RNAs such as microRNAs) on a genomic scale [1]. In this context, microRNAs play a pivotal role.

MicroRNAs (miRNAs, miRs) are a family of small (19–25 nucleotides in length) noncoding RNAs that regulate gene expression by sequence-selective targeting of mRNAs, leading to a translational repression or mRNA degradation, depending on the degree of complementarity between miRNAs and the target mRNA sequences [2–5]. Since their discovery and first characterization, the number of microRNA sequences deposited in the miRBase databases is growing [6–10]. Considering that a single miRNA can target several mRNAs and a single mRNA might contain in the 3′UTR sequence several signals for miRNA recognition, it is calculated that at least 10–40% of human mRNAs are a target for microRNAs [10–13]. Hence, great interest is concentrated on the identification of validated targets of microRNAs.

This specific field of microRNA research has confirmed that the complex networks constituted by miRNAs and RNA targets coding for structural and regulatory proteins lead to the control of highly regulated biological functions, such as differentiation, cell cycle and apoptosis [1–3]. Low expression of a given miRNA is expected to be linked with a potential expression of targets mRNAs. Conversely, high expression of miRNAs is expected to induce low expression of biological functions of the target mRNAs [1–3].

Alteration of microRNA expression has been demonstrated to be associated with human pathologies as well as guided alterations of miRNAs have been suggested as a novel approach to develop innovative therapeutic protocols. MicroRNA therapeutics appears as a novel field in which miRNA activity is the major target of the

Abbreviations: miRNA (miR), microRNA; pri-miRNA, primary miRNA; pre-miRNA, precursor miRNA; RISC, RNA-induced silencing complex; ODN, oligodeoxyribonucleotide; PNA, peptide nucleic acid; LNA, locked nucleic acid; RNA Pol II, RNA polymerase II; TF, transcription factor; mitron, intron containing miRNA sequences; hESC, human embryonic stem cells; EB, embryoid body; UCB, umbilical cord blood; HbF, fetal hemoglobin; ErPCs, erythroid precursor cells; MTH, mithramycin; HPFH, high persistence of fetal hemoglobin; EPO, erythropoietin.

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intervention [14–17]. MiRNA inhibition can be readily achieved by the use of small miR-inhibitor oligomers, including RNA, DNA, DNA analogs (miRNA anti-sense therapy) [14,15]. On the contrary, increase of miRNA function (miRNA replacement therapy) can be achieved by the use of modified, suitably delivered miRNAs mimetics, transfection using recombinant vectors or lentivirus carrying miRNA gene sequences [16,17].

This review article focuses on the involvement of microRNAs in the regulation of gene expression, on the possible role of microRNAs in the onset and development of human pathologies, and on the pharmacological alteration of the biological activity of microRNAs using anti-miR molecules.

2. Biogenesis of microRNAs and drug design

Some miRNAs are encoded by unique genes (intergenic miRNAs) [18–23] and others are embedded into the intronic regions of protein-coding genes (intragenic miRNAs) [24–28]. Examples of intergenic miRNA are miR-210, miR-10a, miR-21, and miR-222/miR-221, which are encoded by unique genes located in the chromosome 11, 17, 17, 6 and X, respectively. The transcription is controlled, as protein-coding genes, by a promoter which is regulated by specific interactions with transcription factors (Fig. 1A). The transcription by RNA polymerase II of these miR genes gives rise to long primary miRNAs (pri-miRNAs) with typical stem-loop structures. These are rapidly processed by the nuclear RNase endonuclease-III Drosha, which, removing the branches, gives rise to precursor miRNAs (pre-miRNA) of around 60–100 nts in length (Fig. 1A).

An example of intragenic miRNA is miR-301. Its genomic sequences are embedded into the intronic regions of ska2 [27]. In this specific case, the transcription of miRNA sequences depends on the cellular promoter of the host gene. The miR sequences follow the splicing pathways giving rise to a "Mirtron" (microRNA/intron) sequence further processed by debranch enzymes to generate a pre-miRNA (Fig. 1B). The microRNA transcription can be controlled by targeting regulatory transcription factors, the microRNA promoter itself, or the promoter of the host gene. An example is that reported by Xi et al., showing that knocking-down of C-EBP-β induces a decrease of the recruitment of this transcription factor on the promoter of the LOC554202 gene (hosting miR-31) and down-regulation of miR-31 [28]. Another

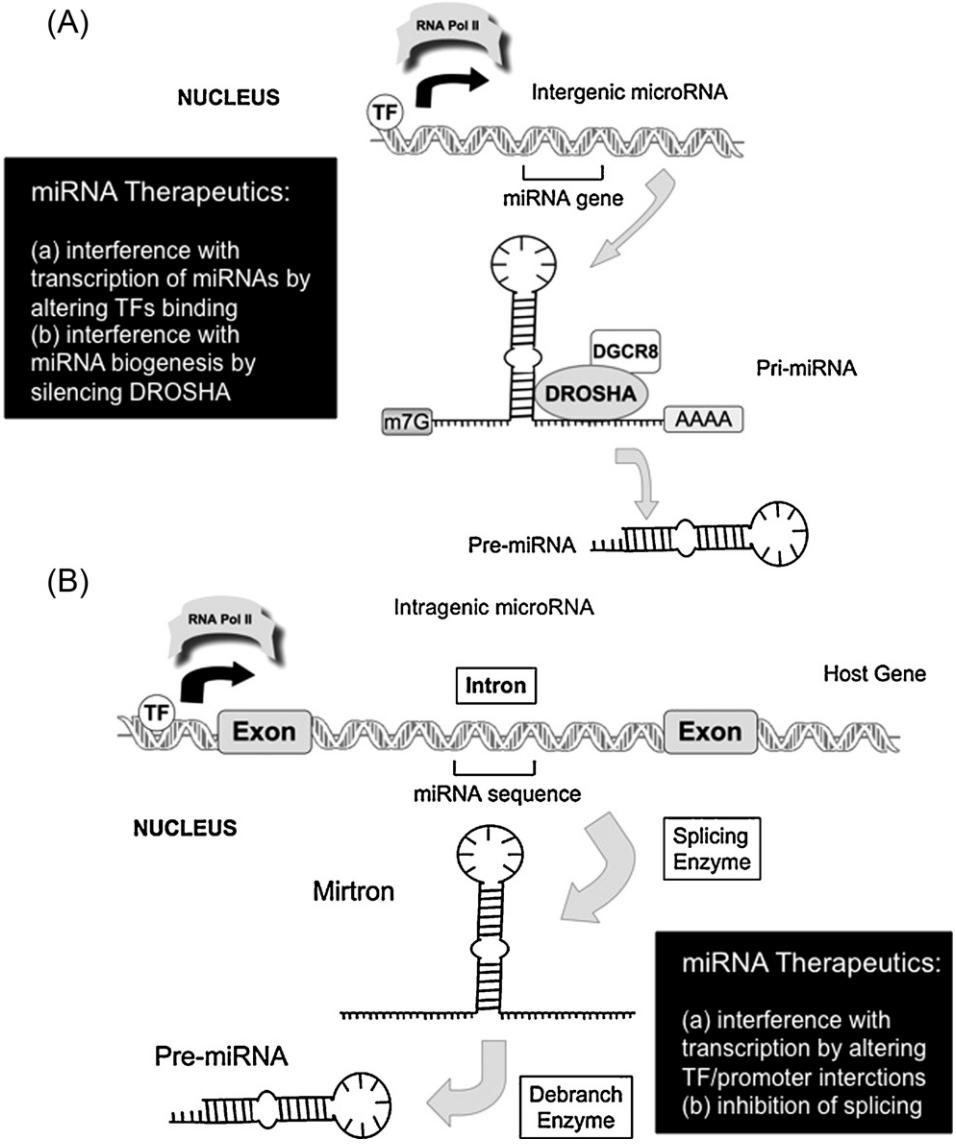

Fig. 1. Biogenesis of miRNAs (I) and possible pharmacological interventions to alter the generation of mature miRNAs. (A) Synthesis of pre-miRNAs by intergenic microRNAs. (B) Synthesis of pre-miRNAs by intragenic microRNAs. For further details on microRNA interference, several reviews are available [60–63].