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RNA oligomers as microRNA production process regulators

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Abstract

The present invention relates to an RNA oligomer, methods for regulating'a microRNA (miRNA) production process and RNA oligomers used as miRNA production process regulators. More precisely, the present invention relates to use of RNA oligomers disrupting a pre-miRNA structure as miRNA production process regulators. Oligomer interactions with a miRNA precursor (pre-miRNA) change the precursor's secondary and tertiary structure. In consequence, the pre-miRNA is not specifically recognised and cleaved by the Dicer ribonuclease, and the specific miRNA is not produced

State of the art

Recently, it has become increasingly clear that the majority of human protein-coding genes are regulated by microRNAs (miRNAs) (Friedman et al., 2009). It means that miRNAs are involved in many biological processes, including developmental timing, growth, differentiation and apoptosis (Ambros, 2003; Kim, 2005; Carthew, 2006). Furthermore, there are several lines of evidence indicating that miRNAs also participate in host-virus interactions (Berkhout and Haasnoot, 2006; Kurzynska-Kokorniak et al., 2009; Jackowiak et al., 2011). Thus, the accurate control of individual miRNA biogenesis is critical for the functions of numerous living organisms, including humans. Even small changes in miRNA production and accumulation can initiate pathological processes, e.g., carcinogenesis, neurodegeneration, immune system or rheumatic disorders (Croce and Calin, 2005). One of the key enzymes involved in the biogenesis of miRNAs in humans is ribonuclease Dicer.

The Dicer ribonuclease belongs to the RNase III family, i.e. to endoribonucleases specifically cleaving double-stranded RNA (dsRNA) molecules. All enzymes in this family are characterized by the presence of one or two ribonuclease domains, referred to as the RNase III domain. RNase III digestion of dsRNAs results in specific products with phosphorylated 5′-termini and 2 nucleotide overhangs at the 3′-ends (Bernstein et al., 2001; Provost et al., 2002; Zhang et al., 2002; Kurzynska-Kokorniak et al., 2015). In miRNA biogenesis, the Dicer ribonuclease is responsible for recognition and cleavage of miRNA

precursors (pre-miRNAs) and release of short, approximately 20-23 nucleotide, duplexes containing miRNAs. According to structural studies, pre-miRNAs adopt a specific secondary structure, called RNA hairpin, including: 1) the double-stranded region (hairpin stem) containing destabilizing elements in the form of internal loops, bulges and mismatches, and 2) the single-stranded region (terminal loop) connecting sequences involved in the stem structure formation. The first reports on miRNA precursor structure significance in the miRNA production process showed that the characteristic structure and the two unpaired nucleotides at the 3′ end play a crucial role in pre-miRNA recognition by the Dicer ribonuclease (Zeng and Cullen, 2003). It was thought that the terminal loop did not influence the miRNA production process much (Han et al., 2006). Han et al. showed, however, that the binding efficiency of some proteins participating in the miRNA production process to pre-miRNAs depends on a terminal loop size. Furthermore, the authors observed that the larger terminal loops present in precursors, the higher efficiency of pre-miRNA processing and thus the higher efficiency of miRNA production occurred. A similar correlation was observed by Zhang X. and Zeng Y. (Zhang and Zeng, 2010). The authors hypothesized that large terminal loops may have an advantageous effect on the flexibility of a precursors' (pri- and pre-miRNA) secondary and tertiary structure, which in turn may facilitate the release of products resulting from either Drosha or Dicer cleavage (miRNA production efficiency increases). Another report published by Michlewski et al. indicated the crucial role of terminal loops present in pri-miRNAs in control of miRNA biogenesis (Michlewski et al., 2008). The authors showed that use of 16-nucleotide RNA oligomers complementary to sequences in pri-miRNA terminal loops (LooptomiRs) prevented binding of key regulatory protein factors to that region, and in consequence, inhibited pre-miRNA production. Moreover, Kloosterman et al. showed that production of selected miRNAs can be inhibited by adding short RNA molecules (and their derivatives) complementary to RNA sequences present in miRNA precursors, being cleavage sites for Drosha and Dicer ribonucleases (Kloosterman et al., 2007).

Our former studies demonstrated that RNA oligomers binding the human Dicer ribonuclease can inhibit that enzyme activity, both in a competitive and in an allosteric way (Tyczewska et al., 2011). Interestingly, our recent results have also revealed that RNA oligomers (and their derivatives) specifically binding to selected miRNA precursors and disrupting their structure, selectively control specific miRNA production by Dicer (Kurzynska-Kokorniak et al., 2013). The present invention relates to an RNA oligomer, methods for regulating a miRNA production process and RNA oligomers used as miRNA production process regulators. More particularly, the present invention relates to use of RNA oligomers disrupting a pre-miRNA structure as miRNA production process regulators. Oligomer interactions with a miRNA precursor (pre-miRNA) change the precursor's secondary and tertiary structure. In consequence, the pre-miRNA is not specifically recognized and cleaved by the Dicer ribonuclease, and the specific miRNA is not produced.

The proposed solution opens a way for designing new, specific therapeutic methods that can be used in treatment of diseases caused by changes in the expression levels of specific regulatory molecules (miRNAs), e.g. in treatment of cancer, or infectious diseases.

Claims

1) RNA oligomer, characterized in that it comprises:
   a) sequences complementary to pre-miRNA single-stranded regions, or
   b) sequences complementary to pre-miRNA double-stranded regions, or
   c) sequences complementary to pre-miRNA single- and double-stranded regions, where the oligomer binds with pre-miRNAs, disrupts a pre-miRNA structure and inhibits the process of miRNA production from pre-miRNA, and:
      i) the RNA oligomer acts bifunctionally: a) as a competitive inhibitor during binding to the Dicer ribonuclease and b) as a molecule disrupting a pre-miRNA substrate structure during binding to the pre-miRNA substrate;
      ii) the RNA oligomer acts as a molecule disrupting a pre-miRNA substrate structure during binding to pre-miRNA substrate, where an oligomer is 12 nucleotide in length;
2) an oligomer, according to claim 1, characterized in that it is selected from:

- RNA oligomers binding with the Dicer ribonuclease,
- RNA oligomers binding with the pre-miRNA substrate,

- RNA oligomers binding with pre-miRNA substrate single-stranded regions,
- RNA oligomers binding with pre-miRNA substrate double-stranded regions,
- RNA oligomers binding with pre-miRNA substrate single- and double-stranded regions,
- RNA oligomers disrupting a pre-miRNA substrate structure,
- RNA oligomers disrupting a pre-miRNA substrate structure so the substrate is not specifically recognised by the Dicer ribonuclease,
- RNA oligomers disrupting a pre-miRNA substrate structure so specific miRNA products are not produced,
- RNA oligomers disrupting a pre-miRNA substrate structure so functional miRNAs are not produced,
- RNA oligomers complementary to pre-miRNA single-stranded regions,
- RNA oligomers complementary to pre-miRNA double-stranded regions,
- RNA oligomers complementary to pre-miRNA single- and double-stranded regions,
- modified oligoribonucleotides, where the modifications can be introduced in the base, sugar or phosphate moieties, where oligomers are 12-nucleotides long or oligomers are selected from RNA oligomers cleaved by ribonuclease Dicer, where the product(s) of the cleavage binds to pre-miRNA;

3) an oligomer, according to claims 1-2, characterized in that it contains sequences complementary to pre-miRNA single- and double-stranded regions, selected from sequences: seq. no. 3 (5′-gggagaaucauaaguag cgca-3′),
   seq. no. 4 (5′-gugagucguugugcugcccauguuaacaguua gcc-3′),
   seq. no. 7 (5′-gaaucuuaacgc-3′),
   seq. no. 8 (5′-ggguaccaccag-3′),
   seq. no. 9 (5′-ggggcagcgcag-3′);
4) a method for control of the miRNA production process, characterized in that it includes stages in which the oligomer, according to claims 1 to 3, reacts with pre-miRNA, disrupts a pre-miRNA structure and inhibits the process of miRNA production from pre-miRNA by the human Dicer ribonuclease;
5) a method, according to claim 4, characterized in that it is a selective and targeted method for miRNA production inhibition;
6) a method, according to claim 4 or 5, characterized in that:
   i) the RNA oligomer acts bifunctionally: a) as a competitive inhibitor during binding to the Dicer ribonuclease, b) as a molecule disrupting a pre-miRNA substrate structure during binding to the pre-miRNA substrate, or
   ii) the oligomer forms a complex with a pre-miRNA and affects its structure so the pre-miRNA is not specifically recognized and cleaved by the human Dicer ribonuclease, and miRNAs are not produced, where the oligomer is 12 nucleotides in length;
7) a method, according to claim 6, characterized in that the sequences complementary to pre-miRNA single- and double-stranded regions are selected from sequences: seq. no. 3, seq. no. 4, seq. no. 7, seq. no. 8, seq. no. 9;
8) use of RNA oligomers, described in claims 1 to 3, disrupting a pre-miRNA structure, as miRNA production process regulators.
9) use, according to claim 8, in which sequences complementary to pre-miRNA single- and double-stranded regions are selected from the following sequences: seq. no. 3, seq. no. 4, seq. no. 7, seq. no. 8, seq. no. 9.

Current status

1) International Application No. PCT/PL2013/000024: in the written opinion of the International Preliminary Examining Authority it was stated that RNA oligomers (of specified length) binding to pre-miRNAs single stranded regions inhibiting the production of specific miRNAs could be considered novel.
2) Application in USPTO (date: 21. 07. 2014).

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