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The correlation of structural features of mature miRNAs with their biological function

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Abstract

miRNAs are short non-protein coding RNAs, being though a crucial regulators of gene expression of up to 90% of human genes (Friedmann et al., 2009; Guo et al., 2010; Perron and Provost, 2010; Bartel, 2009; Esquela-Kerscher and Slack, 2006; Bartel, 2006; Kozomara and Griffiths-Jones, 2011; Selbach et al., 2008). These tiny RNA molecules tune cell growth, tissue differentiation, cell proliferation, embryonic development, apoptosis and cellular signaling (Gaur et al., 2007; Godlewski et al., 2012; Kim et al., 2010; Cui et al., 2006). Even slight shift in miRNA level could lead to significant changes of transcriptome, and in a result of cell phenotype. In the last decade, over 30 000 mature miRNA sequences were deposited in miRBase (Griffiths-Jones et al., 2006). The function of many of them have been found and anti-miRNA tools, as potential therapy approach have been designed. Despite of an enormous data of miRNA, there are still many questions concerning miRNA function to be solved. Following Francis Crick's famous statement "If you want to understand function, study structure", we were looking for structure of mature miRNAs (Belter et al., 2014).

The miRNA structure

Using specific nucleases (RNase T1, RNase V1 and nuclease S1), NMR, UV/Vis, CD spectroscopies, we showed miR-21, miR-93 and miR-296 secondary structure. We found that at low cellular concentration of miRNAs, they form mainly hairpins (Belter et al., 2014). The observations are consistent with our and previous *in silico* studies, which have also shown that above 70% of human (Maiti et al., 2010), mammalian (Adhikary et al., 2011) and plants (Das et al., 2011) miRNAs may fold into hairpin structures and almost 70% could potentially form self-aggregated homoduplexes.

Looking for strong evidences that miRNA are structured molecules, we compared short RNA molecules, which form well-characterized structures with miRNAs. We noticed a high homology of their sequences (Fig. 1). 5′-end of has-miR-607 and 3′end of T-stem and TψC loop of human $tRNA^{Cys}$ have identical sequence on the length of 12 nucleotides. High sequence homology was also observed for hsa-miR-1193 and human 5S rRNA, has-miR-1290 and $minihelix^{Ala}$, purine-rich has-miR-3960 and GGAGGAGGAGGA sequence (Fig. 1). All identified RNAs of high homology to mentioned miRNAs are short RNAs, which form highly ordered structure. It is known that, their functions are determined by their structures. Based on that observation, we postulate that mentioned miRNAs may form structural motives of functional importance. Even, the commonly accepted model of miRNA-guided RNA down-regulation suggests that mature miRNA, being a part of miRISC complex, targets mRNA in the nucleotide sequence-specific manner (Liu et al., 2004; Hutvagner et Zamore, 2002). Therefore our results indicate that miRNA function may depend not only on their sequence but also structure (Fig. 2).

Functional consequences of miRNA structure

Previously, it has been reported that the secondary structure of siRNAs, short miRNA-like RNAs, influences the efficiency of siRNA-mRNA interaction. Unstructured siRNAs confer stronger silencing abilities than structured guide siRNAs (Khvorova et al., 2003). By analogy to siRNA, we thought the stable miRNA structures may influence miRNA-mRNA complex formation. Thermodynamic of RNA-RNA interactions depend both on the structure and accessibility of mRNA target sites and structure of regulatory miRNA as well. The secondary structure of miRNA can have a conformational role to modulate miRNA-mRNA interactions and thus can explain the dif-

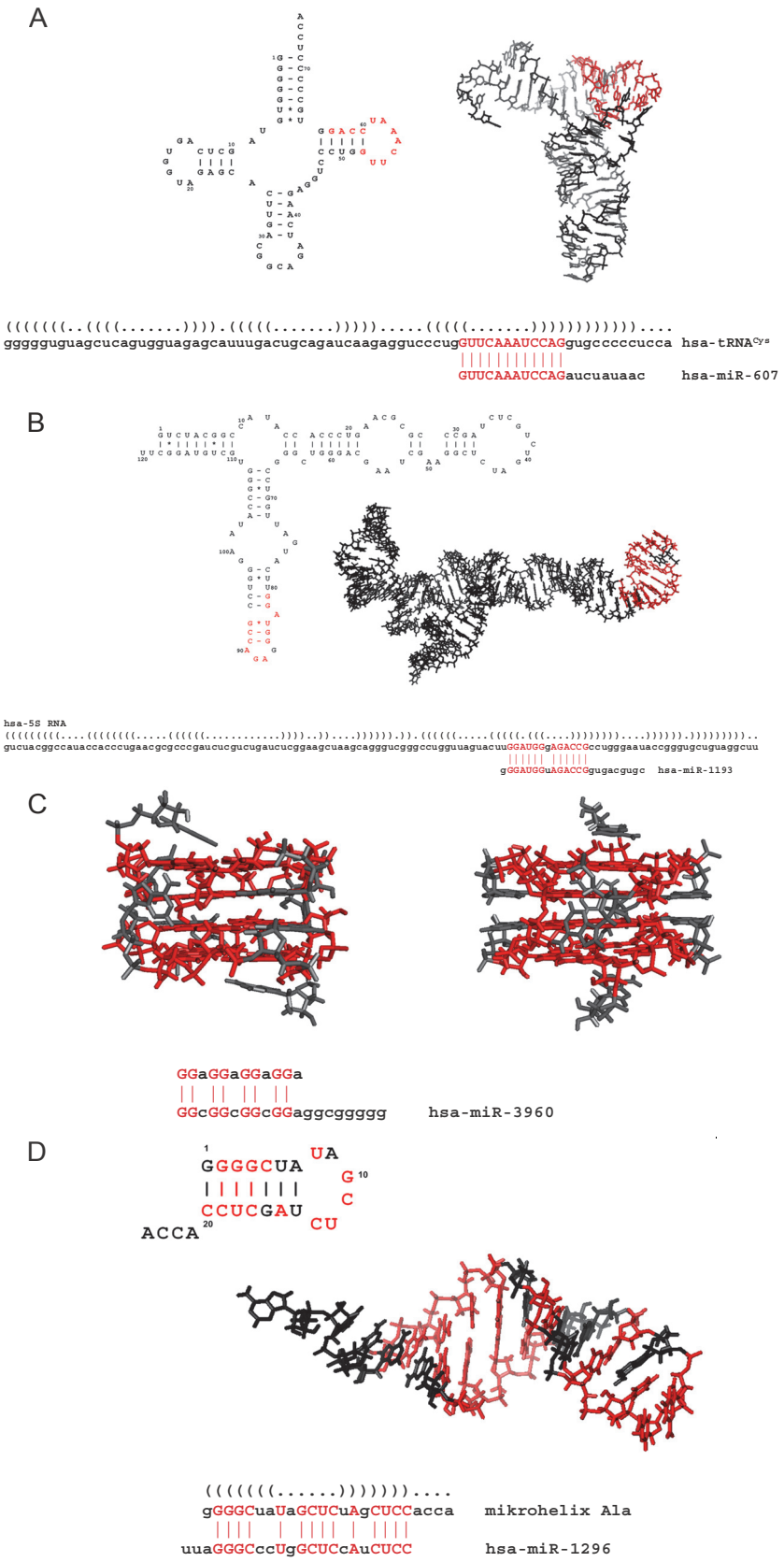

Fig. 1. Sequence similarity of some miRNAs and other short RNAs. A) hsa-tRNA^{Cys} and hsa-miR-607, B) hsa-5S RNA and hsa-miR-1193, C) GGAGGAGGAGGA quadruplex and hsa-miR-3960, D) microhelix^{Ala} (Musier-Forsyth and Schimmel, 2002) and hsa-miR-1296. The quadruplex structure has been determined with NMR spectroscopy (2RQJ) (Mashima et al., 2009), tRNA^{Cys}, 5S rRNA oraz microhelix^{Ala} using RNA Composer (Popenda et al., 2012)

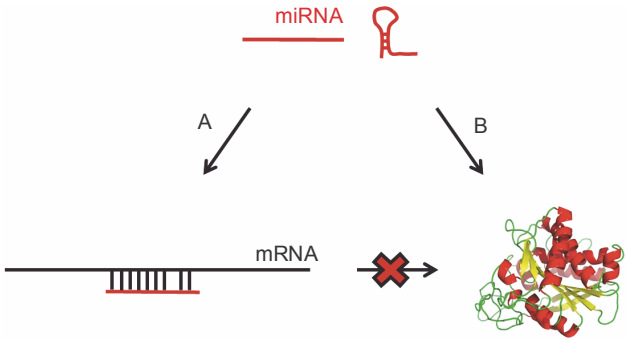

Fig. 2. RISC-dependent A) and RISC independent B) function of miRNA. Mature miRNA incorporated into the miRNA-induced silencing complex (RISC) targets mRNA in a specific manner causing its degradation or repression of its translation (Liu et al., 2004) – A. The structure of miRNA suggests that miRNA may function also beyond RISC complex – B

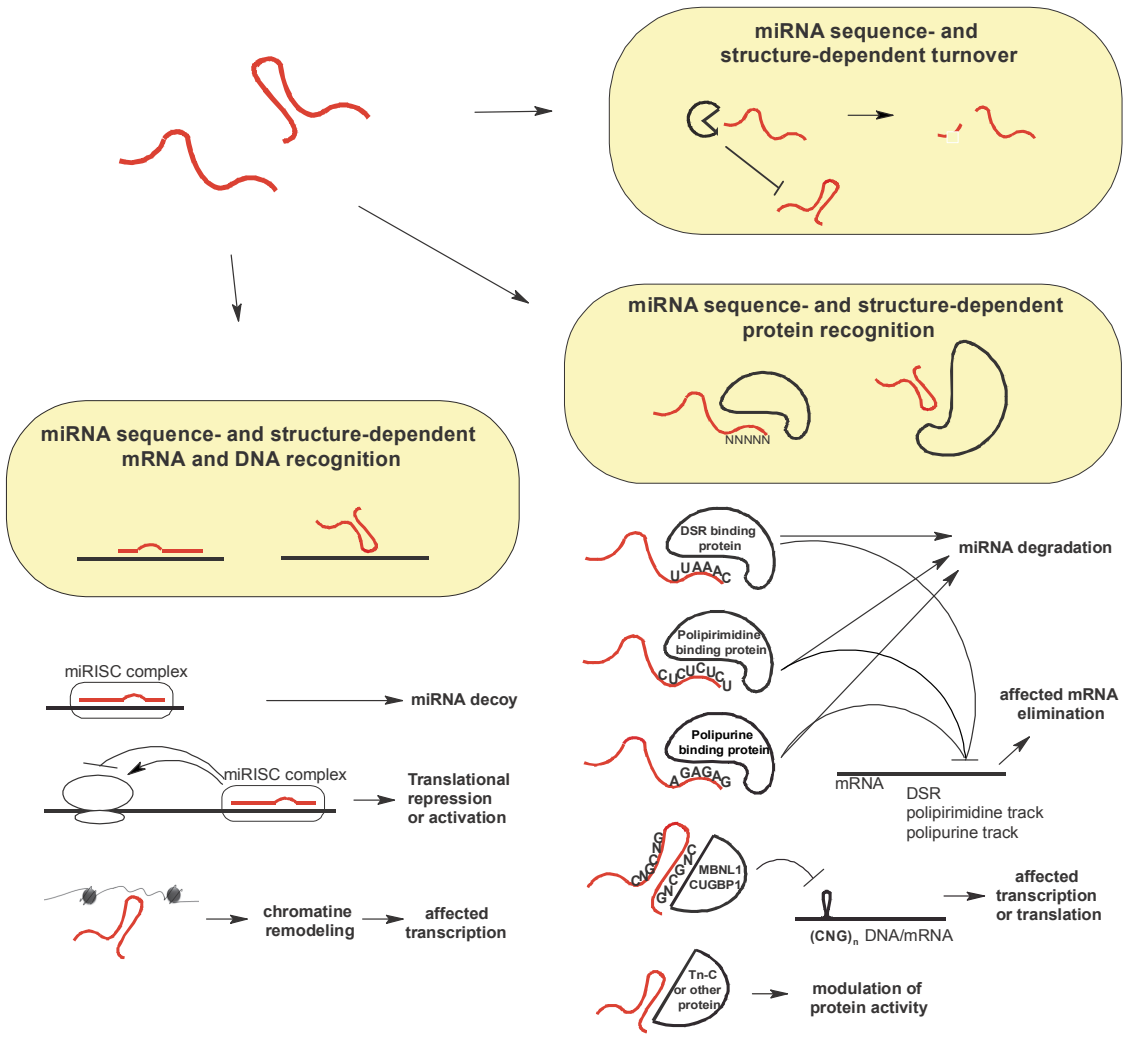

Fig. 3. The sequence- and structure-dependent function and turnover of miRNAs. The commonly accepted model of miRNA-guided RNA down-regulation suggests that mature miRNA targets mRNA in a sequence specific manner causing its degradation and repression or activation of its translation. The secondary structure of miRNA can have a conformational role to modulate miRNA-mRNA interactions. Some sequence motifs, e.g. polipurine and polipirimidine track identified within miRNA and structural versatility of miRNA suggest a variety of functions beside widely accepted mRNA recognition, degradation and destabilization. The structure of miRNAs affects also their turnover

ferent degree of genetic regulation for the specific miRNA involved in regulation process (Fig. 3). It has been shown that bases 2-8 of 5′ end of the miRNA are crucial to initiate mRNA binding. Thus, we suppose that miRNAs, which seed region is not knotted in secondary structure more easily interact with target mRNA than these, which seed region is a part of hairpin stem (Belter et al., 2014).

Interestingly, within miRNAs, we identified numerous sequence motifs responsible for hairpin formation and recognized by protein, such as MBNL-1 and CUG-binding proteins (Belter et al., 2014) – Figure 3. For example miR-320, miR-709 and miR-233 may recruit proteins, which direct chromatin remodeling (Zardo et al., 2012; Adilakshmi et al., 2012). miR-888 and miR-146a have been shown to bind to the nucleocapsid domain of the Gag protein, the main structural component of HIV-1 virions and interfere with viral-RNA-mediated Gag assembly and viral budding at the plasma membrane (Chen et al., 2014). We showed also a high structure similarity of some miRNAs hairpins to aptamers, e.g. anti-Tn-C aptamers (Belter et al., 2014) and suggested that miRNAs, as RNA aptamers, may be prone to interact with proteins and consequently directly regulate their activity.

Additionally, we noticed that the higher-order motifs within miRNA may extended turnover time of these miRNAs (Gantier et al., 2011). miRNAs, which fold into knotty secondary structures are more stable and miRNA stability correlates with ΔG of their structure. We observed that the half-life of miRNA is longer for those of them, which ends are knotted in hairpin stem, e.g. like in case of miR-296 (Belter et al., 2014). We think that the higher-order motifs within miRNA and the differences of secondary structure energy may explain the different turnover time of miRNAs.

The above results strongly support our idea that miRNAs may function beyond the RISC complex. It suggests the existence of novel mechanism regulating miRNA function through fine-tuning of steady-state miRNAs level.

Conclusions

There are many data which are not consistent with a high specificity of miRNA. The current model of their action does not explain how such a short nucleotide sequence of "seed" region can determine the specificity of miRNA action. Within the cell miRNAs may exist as free molecules showing structural versatility. This may determine a variety of functions beside widely accepted mRNA recognition, degradation and destabilization. Thus determining miRNAs structure may help understand their functions also these beyond RISC complex. Therefor we would like to underline the importance of new structural data of miRNA as they may provide new clues of orchestred miRNA-dependent gene regulation.

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