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Molecular Diagnostics Using miRNA

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Introduction

MicroRNAs (miRNAs) are a type of small RNA that averages 22 nucleotides in length. They are non-coding RNA molecules that regulate gene expression in many organisms, including the human genome. miRNAs play important roles in many cellular and developmental processes of eukaryotic organisms by patrolling the genome and transcriptome. Lin-4, the first discovered miRNA, regulates developmental timing in the nematode Caenorhabditis elegans by blocking gene lin-14, which encodes proteins required for development after the larvae stage. A few years later, another gene, let-7, was discovered, which encodes a 21-nts-long molecule RNA important in the development of the same species. Since then, the number of known miRNAs has grown significantly [1].

Description

Given that small changes in miRNA levels can disrupt the regulation of various target genes, proper control of miRNA biogenesis is critical for maintaining cell homeostasis. To produce functionally mature miRNAs, miRNA biogenesis is subjected to complex regulation at both the transcriptional and post-transcriptional levels. The majority of miRNA genes are found in protein-coding regions of the genome or within introns of protein-coding genes. miRNA expression is regulated independently of host gene expression via their own promoters. miRNA genes are transcribed by RNA polymerase II and, in some cases, RNA polymerase III [2].

miRNA biogenesis is a series of sequential processes that result in canonical or non-canonical pathways (Figure 1). Most miRNAs, however, are known to be synthesized via a canonical pathway. miRNAs go through two sequential maturation steps in canonical pathways. The first step involves the nucleus enzyme Drosha (RNase III enzyme) and the cytoplasm enzyme Dicer. Pri-miRNAs are converted into pre-miRNAs in the nucleus. The nuclear protein DGCR8 (Di-George Syndrome Critical Region 8) recognises the RNA stem-loop and binds to Drosha, forming a microprocessor complex that cleaves pre-miRNA to form pre-miRNA. Pre-miRNAs are 65 nts long and are observed to have a two-nucleotide-long 3′ overhang [3].

Exportin 5, a transmembrane protein, recognises 3′ overhang ends on pre-miRNAs and transports them to the cytoplasm with the cofactor RanGTP. Pre-miRNAs in the cytoplasm are processed by Dicer after they have been exported from the nucleus by the exportin 5/RanGTP complex. Dicer contains two RNAse III catalytic domains, a double-strand RNA-binding domain and the PAZ domain, which bind the 3' end of small RNAs, as well as an RNA helicase/ATPase domain. Dicer interacts with cofactor TRBP via its helicase domain (TAR-RNA binding protein). Although TRBP does not appear to be required for Dicer-mediated pre-miRNA processing, the Dicer-TRBP complex removes the terminal loop of the pre-miRNA to release a mature double-strand miRNA (dsRNA).

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Generated dsRNAs are named miRNA/miRNA* (miRNA-guide strand, miRNA*-passenger strand) or miR-3p/miR-5p, referring to the functional miRNA's direction. The 3p strand is formed from the 3′ end of the pre-miRNA hairpin, whereas the 5p strand is formed from the 5′ end. The mature miRNA guide strand is one of the two strands of dsRNA and the complementary passenger strand is the other. The origin of mature miRNA from either the 5p or 3p strand is determined by many factors, including cell type, cellular environment, thermodynamic stability and many others. The guide strand, in general, has lower 5′ stability and it is introduced into the RISC complex (RNA-induced silencing complex), which contains the Argonaute (AGO 1-4) protein family and functions as a post-transcriptional regulator [4,5].

Conclusion

Given the importance of the RISC complex in the execution of miRNA-based silencing, the function of miRNA is to direct the RISC complex to complementary sequences on target mRNAs. miRNAs typically interact with the complementary 3′ UTR region of target miRNAs to cause mRNA degradation and translational inhibition. miRNAs regulate the expression of many proteins, including tumour suppressors and protooncogenes and thus become oncogenes and tumour suppressors. Because the same miRNA can have different targets in different tissues, its function will vary depending on the intracellular milieu and the set of proteins for which its translation is modulated. As a result, the same miRNA can function as both a tumour suppressor and an oncogene in different cancers.

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Conflict of Interest

There are no conflicts of interest by author.

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