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Viral protein-based bioanalytical tools for small RNA biosensing

Susana Campuzano *, María Pedrero, José M. Pingarrón *

Departamento de Química Analítica, Facultad de CC. Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain

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ABSTRACT

Small RNAs, in particular microRNAs (miRNAs), are important regulators of gene expression and many pathological conditions, which have now been definitively linked to the development of cancer. Therefore, accurate and sensitive quantification of miRNAs may result in correct disease diagnosis, establishing these small noncoding RNA transcripts as valuable biomarkers. Aiming at overcoming some limitations of conventional quantification strategies, viral proteins offer interesting alternatives to small RNA biosensing. In this review, an up-to-date critical account of recombinant viral protein-based strategies, involving different readout techniques, for miRNA determination is provided. The variety of viral protein-based strategies for miRNA quantification that have appeared in recent years highlights this growing research field, which has not only several challenges but also great opportunities to develop sensitive, reliable, robust, and cost-effective small RNA biosensing methodologies.

Contents

1. Introduction

First discovered in 1998, RNA interference (RNAi) is a biological process in which small RNA molecules inhibit gene expression, typically by causing the destruction of specific messenger RNA (mRNA) molecules [1]. Two types of small RNA molecules – microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNAi. These small RNAs can bind to other specific mRNA molecules and either increase or decrease their activity, for example, by preventing protein production by an mRNA. RNAi plays an important role in cell defense against parasitic nucleotide (nt) sequences – viruses and transposons – and also influences cell development.

The cellular response to double-stranded RNA (dsRNA) is a critical component of the innate immune response to RNA viruses in eukaryotes. An integral component of the dsRNA response is the evolutionarily conserved RNA-silencing response, whereby dsRNAs are converted by the RNase III enzyme Dicer into short duplex RNAs of approximately 21-nt length, known as siRNAs, which are used to target and repress the virus [2]. Each siRNA is unwound into two single-stranded RNAs (ssRNAs), the passenger and the guide strands. While the passenger strand is being degraded, the guide strand is incorporated into the RNA-induced silencing complex (RISC) [3]. Although this reaction is highly potent, RNA viruses have evolved mechanisms of inhibiting this pathway, using silencing suppressor proteins, thereby allowing viral propagation in their host [2].

* Corresponding authors. Tel.: +34 913944315; Fax: +34 913944329.
E-mail addresses: susanacr@quim.ucm.es (S. Campuzano); pingarro@quim.ucm.es (J.M. Pingarrón).

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For example, Tombusviruses, a family of ssRNA viruses of plants, use these RNA-binding proteins as suppressors of the RNA silencing pathway during infection.

By exploiting the high affinity of these viral silencing suppressor proteins for small RNAs, highly promising strategies for siRNA and miRNA biosensing have been recently developed. A brief overview of the most relevant approaches in this rapidly advancing field is presented. The fundamentals of the different developed strategies are discussed, highlighting some examples, future prospects, and challenges.

2. Types of small RNAs

As commented previously, the process of RNAi can be modulated by either siRNA or miRNA, but with subtle differences. Both are produced by Dicer-mediated cleavage of longer dsRNA precursors and incorporated into the RISC. However, siRNA is an exogenous dsRNA that is taken up by cells, or is introduced by transfection, whereas miRNA is an endogenous noncoding ssRNA. siRNAs are synthesized from ds segments of matched mRNA via RNA-dependent RNA polymerase. Conversely, miRNAs are synthesized from an unmatched hairpin-shaped RNA precursor segment. Furthermore, miRNAs cluster in "families" closely related to the sequence is concerned or as individual units. siRNAs are often derived from repetitive DNA sequences and associated transposons and centromeres, forming heterochromatin structures. Furthermore, none of the genes encode for siRNAs, whereas miRNAs are encoded by specific miRNA genes as short hairpin primary transcripts (pri-miRNAs) in the nucleus. While the main mechanism of action of siRNA is mRNA cleavage [4], the main function of miRNA is the inhibition of protein synthesis by blocking mRNA translation. miRNAs are short noncoding RNAs that regulate gene expression [5] in mRNAs of one-third of genes [6]. One miRNA can bind to one or more mRNAs, and some mRNAs have multiple binding sites for different miRNAs belonging to the same family [7]. Another difference is that, in animals, siRNA typically matches perfectly with its target mRNA, whereas miRNA often contains mismatches, G:U base pairs, and single-nt bulges, and it can inhibit the translation of several different mRNA sequences as a result of this imperfect pairing [8].

2.1. Small interfering RNAs (siRNAs or silencing RNAs)

siRNAs are 20 – 25-base-pair dsRNA molecules that play an important role in the RNAi pathway, whereby they interfere with the expression of specific genes with complementary nt sequences. siRNAs trigger the destruction of the target mRNA with which they share complementarity, thus impairing the process of translation, although transcription continues as usual. As a result, the overall protein content of the target gene is reduced, but the mRNA is produced and degraded at the same pace and gene expression is not altered unless the homeostatic system of the cell alters it in response to stress generated by siRNAs. They also act in RNAi-related pathways, for example, as an antiviral mechanism, or in shaping the chromatin structure of a genome. siRNAs and their role in posttranscriptional gene silencing (PTGS) in plants were discovered in 1999 by David Baulcombe's group [9]. Shortly after, Tuschl et al. reported that synthetic siRNAs could induce RNAi in mammalian cells [10] which led researchers to focus on RNAi for basic biomedical research and drug discovery and development.

siRNAs have a well-defined structure: a short dsRNA with phosphorylated and hydroxylated 5′ and 3′ ends, respectively, with two overhanging nt's. The production of siRNA from long dsRNAs and small hairpin RNAs is catalyzed by the Dicer enzyme. As any gene can be knocked down by a synthetic complementary siRNA, siRNAs are crucial for gene function and drug target validation.

2.2. Micror RNAs (miRNAs or miRs)

Mature miRNAs are evolutionally conserved, endogenously expressed, and 21–25-nt small non-protein-coding ssRNAs with two-nt 3' overhanging ends and 5' phosphate groups. They have recently emerged as key regulators of gene expression via repression of mRNA translation or mRNA degradation in a wide variety of animals, plants, and viruses [11].

The biogenesis of miRNAs is a multiple-step process before they are exported from the nucleus to the cytoplasm [12]. Initially, miRNAs are transcribed in the nucleus from intragenic or intergenic regions by RNA polymerase II to form 1 – 3-kb stem-loop structures known as pri-miRNAs, which are cleaved in the nucleus by the RNase III enzyme Drosha and the dsRNA-binding protein Pasha into the pre-miRNAs (70 – 100-nt stem-loop structures). These pre-miRNAs are then transported to the cytoplasm by exportin-5, to be further cleaved (by removing the loop) by the RNase III enzyme Dicer into mature 18 – 24-bp dsmiRNAs. After strand separation, one of the strands becomes a mature miRNA incorporated into RISC, whereas the other, called the passenger miRNA strand, is degraded rapidly [13]. miRNA-bonded RISC can target protein-coding mRNAs either for translational inhibition or for destruction; this choice is governed by the degree of mismatch between the miRNA and its target mRNA, degradation being the outcome for the best-matched targets. As miRNAs can inhibit the translation of imperfectly matched targets, each miRNA may target multiple genes and a given target may be regulated by several miRNAs. Although the interplay between mRNA and miRNA is vital for gene expression regulation, studies into the role of this interplay in disease have only just begun. The initial stem-loop configuration of the primary transcript provides structural clues to determining the genomic sequences of candidate miRNA genes [14].

miRNAs, identified in normal and malignant cells, are predicted to regulate at least one-third of all human genes. They play critical regulatory roles in various physiological (cell development, cell differentiation, metabolism, and immune system) and pathological (oncogenesis) processes [13]. Deregulated miRNA levels, indicative of abnormal gene regulation, have been associated with numerous illnesses including cancer, cardiovascular disease, neurological disorders, and diabetes [11]. miRNAs act to promote or repress cell proliferation, migration, and apoptosis during development, all of which represent altered processes in cancer. Thus, miRNAs can act as both oncogenes and tumor suppressors. Based on these findings, miRNAs are considered reliable biomarker candidates for the detection, diagnosis, and prognosis of cancer [15]. Thus, miRNAs provide the oncologist with a potentially powerful new tool to diagnose and treat neoplastic diseases. Consequently, the ability to detect and quantify the expression levels of one or more interesting sets of miRNAs reliably is of great clinical relevance and is expected to have a significant impact on health care [11].

3. Conventional methods for miRNA detection and quantification

The analysis of miRNAs is very challenging due to their unique characteristics: small size, low abundance, and sequence similarity among family members [16]. The small size of miRNAs greatly complicates the polymerase chain reaction (PCR) or hybridization-based miRNA assays, because primers that are shorter than usual would be required, which affects the PCR efficiency. In addition, the labeling of a short probe for selective detection of miRNAs in the case of hybridization approaches is not an easy task. Moreover, the melting temperature of the resulting duplex is low, which sharply decreases the hybridization stringency, increases the cross-hybridization risk, and the probability of false-positive signals. Furthermore, miRNA concentrations can be as low as a few