Detection of microRNAs

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MicroRNAs (miRNA) include a set of short, noncoding proteins and small RNA molecules with a length of 20–24 nt, generated by the RNase-III-type enzyme Dicer from an endogenous transcript that contains a local hairpin structure. After unwinding, the miRNA forms part of the RNA-induced silencing complex (RISC) assembly and causes translational repression or mRNA degradation.

Keywords: CRISPR-Cas13 technology ; miRNA detection ; biosensing ; signal amplification

1. Overview

MicroRNAs (miRNAs) have a prominent role in virtually every aspect of cell biology. Due to the small size of mature miRNAs, the high degree of similarity between miRNA family members, and the low abundance of miRNAs in body fluids, miRNA expression profiling is technically challenging. Biosensors based on electrochemical detection for nucleic acids are a novel category of inexpensive and very sensitive diagnostic tools. On the other hand, after recognizing the target sequence, specific CRISPR-associated proteins, including orthologues of Cas12, Cas13, and Cas14, exhibit collateral nonspecific catalytic activities that can be employed for specific and ultrasensitive nucleic acid detection from clinically relevant samples. Recently, several platforms have been developed, connecting the benefits of enzyme-assisted signal amplification and enzyme-free amplification biosensing technologies with CRISPR-based approaches for miRNA detection. Together, they provide high sensitivity, precision, and fewer limitations in diagnosis through efficient sensors at a low cost and a simple miniaturized readout. This review provides an overview of several CRISPR-based biosensing platforms that have been developed and successfully applied for ultrasensitive and specific miRNA detection.

2. MicroRNAs

MicroRNAs (miRNA) include a set of short, noncoding proteins and small RNA molecules with a length of 20–24 nt, generated by the RNase-III-type enzyme Dicer from an endogenous transcript that contains a local hairpin structure [1][2]. After unwinding, the miRNA forms part of the RNA-induced silencing complex (RISC) assembly and causes translational repression or mRNA degradation [3].

The primary signature of miRNAs is their master role in repressing the expression of multiple protein-coding genes at the transcriptional, post-transcriptional, and translational levels by binding to the promoter regions, 3′ untranslated region (3′-UTR), 5′-UTR, and the coding regions of target sequences [3][4][5].

Recently, the universality of miRNAs in research has continuously grown, as the presence or dysregulation of distinct miRNAs can indicate specific pathologic conditions. In some cases, several miRNAs have been identified as oncogenic elements or as tumor suppressors, and in other cases, they have been shown to participate in diverse pathways, having different effects on cell survival, growth, and proliferation depending on the cell type and the pattern of gene expression [6][7]. For example, miR-17 has been selected as a proof-of-concept model target for different biosensors because of its role in many diseases, including breast cancer [8], pulmonary hypertension [9], and Mantle cell lymphoma [10]. Therefore, miRNAs offer new possibilities for potential diagnostic and prognostic tools in miRNA-associated diseases such as cancer [4][7]. Considering the master role that miRNAs play in regulating critical biological processes and that their ability to repress their targets crucially depends on their expression levels [3][5][11], it is highly desirable to construct specific and ultrasensitive methods for the detection of miRNAs showing low expression levels.

3. Conclusions

MiRNAs are becoming the biomarkers in clinical diagnostics of multiple diseases due to their dysregulation associated with many different conditions, such as cancer [12][13], dementia [14], and cardiovascular diseases [15][16]. Thus, miRNA

detection can be crucial for an early accurate initial diagnosis, evaluating responses to effective treatment, and improving the patient's survival probabilities.

Designing a strategy that could broadly target and detect miRNA sequences (particularly with low expression levels) would be an invaluable resource. In this regard, in diagnostics, synthetic biology employs forward-engineering approaches that are typically focused on building sensors coupled to a measurable output to create new molecular functions [17].

Owing to the inherent advantages of colorimetric and electrochemical transduction methods and their excellent compatibility with the CRISPR-Cas13 technology, the detection of miRNAs through these novel biosensors can potentially be scaled in high-performance devices at low cost with a simple miniaturized readout [18].

It should be noted that the detection limit of CRISPR-Cas-mediated amplification-free nucleic acid detection is usually at the pM level [19], and recently, several CRISPR-Cas-based biosensors have improved the sensitivity to aM, or even zM, by target amplification [20][21]. In this respect, all the biosensors described here give us rapid, real-time, precise, and authentic information about miRNA molecule detection with desirable sensitivity and specificity. Importantly, these approaches are cheaper and cost-effective in sample and reagent consumption than the current gold standard RT-qPCR for miRNA detection.

The versatility is unprecedented for a single protein. Moreover, simply by changing the spacer sequence of crRNAs, it is possible to detect any miRNA sequence, indicating the excellent versatility of these proposed biosensing platforms. In recent years, this programmable nature of the CRISPR-Cas13 system has allowed the scientific community to move remarkably fast to develop an innovative diagnosis for a wide variety of human miRNAs, with important implications in the clinic.

However, the existence of off-target activity is not surprising, as molecular interactions are never perfectly specific [22]. Thus, the possible limitations of the CRISPR-Cas13 system could include: (1) RNA recognition of small RNA target sequences (< 22 nt) because crRNAs need to be long enough for binding [23]; (2) As the fidelity of the CRISPR-Cas13 system is directly related to the tolerance of RNA mismatches, a higher mismatch tolerance of Cas13 effectors could propitiate off-target activity [24]; (3) Added to this, the requirement of specific PFS for Cas13 effectors may be hard to satisfy; and (4) RNA segments derived from effective ssRNA cleavage mediated by Cas13 could be toxic in eukaryotic cells [23][25]. Therefore, further detailed structural studies and functional validation of Cas13 effectors in cells will be vital for defining their mechanistic differences and operational efficiency [26].

Nevertheless, although we have not yet exploited the full potential of CRISPR-Cas13-based platforms for miRNA detection and monitoring in clinical settings, this technology represents a revolutionary advancement in engineering sensors as minimalist strategies.

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