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microRNA biosensors: Opportunities and challenges among conventional and commercially available techniques

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ABSTRACT

As being the most extensively studied, non-coding, evolutionary conserved, post-transcriptional gene regulators of genome, microRNAs (miRNAs) have taken great attention among various disciplines due to their important roles in biological processes and link with cancer. Due to their diagnostic value, there have been many conventional methods used in detection of miRNAs including northern blotting, quantitative real time PCR (qRT-PCR) and microarray technology besides novel techniques based on various nanotechnology approaches and molecular biology tools including miRNA biosensors. The aim of this review is to explain the importance of miRNAs in biomedical field with an emphasis on early cancer diagnosis by overviewing both research based and commercially available miRNA detection methods in the last decade considering their strengths and weakness with an emphasis on miRNA biosensors.

1. Introduction

1.1. miRNA: background

Ever since its discovery dates back to 1990s, researchers have paved a long way in the field of microRNAs (miRNAs), a large class of small (ranging in length 18–25 bp) non-coding RNA that were unappreciated before the investigation of their roles in lin-4 and lin-14 genes by Ambros and his colleagues who showed the posttranscriptional regulation of lin-14 protein synthesis by a 22-nt transcript (Lee et al., 1993). After its discovery several follow-up work and discovery also summarized by Fig. 1 have enlightened the action of mechanism of these gene regulators and lead miRNAs to burst onto medical diagnosis field. Among all other functions they possess, the main function is believed to be post-transcriptional regulation of proteins. The abundance of miRNAs in both invertebrates and vertebrates has been proven in 2001. According to the current database (http://www.mirbase.org), there are total 1881 annotated human miRNA precursor genes that processes 2588 mature miRNAs. Although miRNAs abundant in tissues, it has also been shown that, there are also circulating miRNAs in body fluids such as plasma, urine, saliva, peritoneal fluid, pleural fluid, seminal fluid, tears, amniotic fluid, breast milk, bronchial lavage, cerebrospinal fluid, and colostrum. The amount of total RNA found in plasma is in 6–300 ng/mL and miRNA fraction is known to be only a few percent of total RNA (Weber et al., 2010). Although the exogenous miRNAs are comparatively small amount in plasma, they are also stable as endogenous miRNAs which exist either inside micro vesicles in an encapsulated form or bound to RNA binding proteins such as Ago2 to be protected from degradation (Vickers et al., 2011).

1.2. miRNA biogenesis

Although miRNAs have various critical roles in wide range of biological processes including embryonic development (Virant-Klun et al., 2016), proliferation, apoptosis, hematopoiesis and link between their expression to some human diseases, genetic disorders and cancer onset, the underlying principle of their function is RNA-mediated gene silencing through RNA interference (RNAi)-like pathways (Bhaskaran and Mohan, 2014; Farazi et al., 2013; Montagner et al., 2014; Moreno-Moya et al., 2014; Piubelli et al., 2014). Initially, a large piece of mRNA includes the specific miRNA stem-loop structure that is also known as pri-miRNA is transcribed by RNA polymerase II in the nucleus. After transcription, Drosha, an RNAse III endonuclease cleaves pri-miRNA into stem loop structure named as pre-miRNA. After its transportation from nucleus to cytoplasm via Exportin-5 protein, pre-miRNA is cleaved by another RNAse III enzyme, Dicer and maturates to miRNA (Bhaskaran and Mohan, 2014; Dong et al., 2013; Moreno-Moya et al., 2014; Zhuo et al., 2013). miRNAs regulate genes by interfering with intracellular messenger RNA (mRNA) either directly through cleavage mRNA or indirectly through repression of translation.

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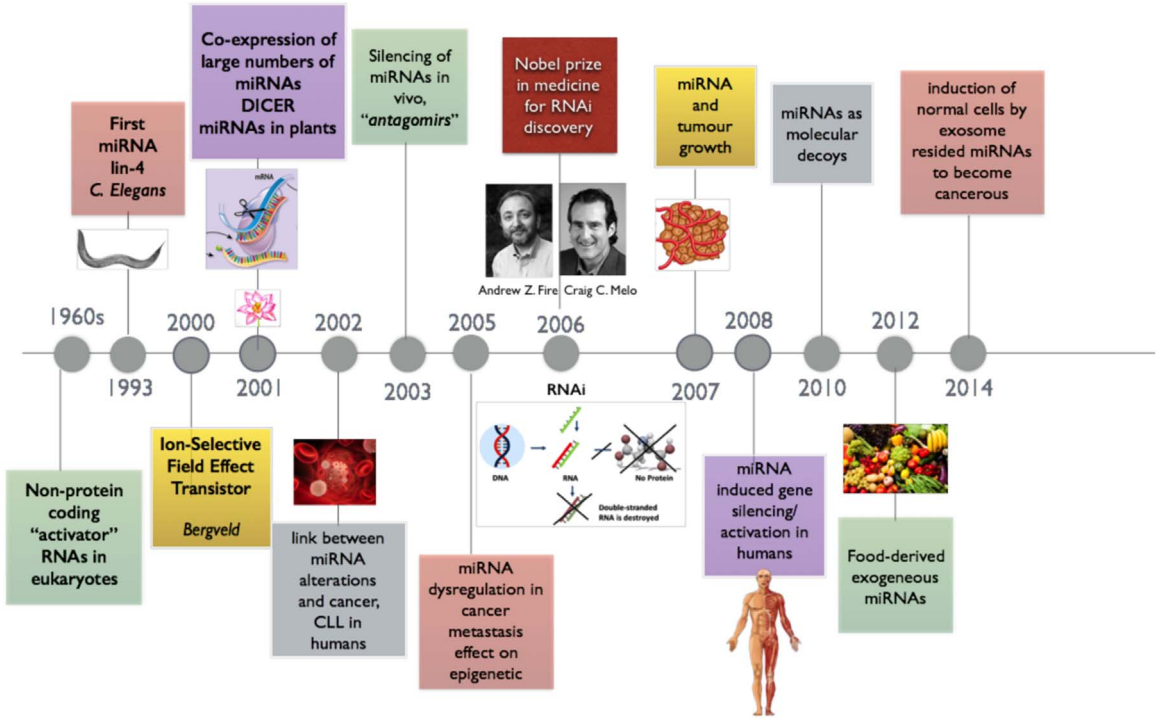

Fig. 1. Advances in miRNA research as a timeline.

In both cases, miRNA base pairs with its target mRNA from its 3′ untranslated region (3′ UTR). Depending on the complementarity – complete or incomplete, between two, target mRNA is either degraded or its translation is blocked.

1.3. miRNAs as biomarkers

To date, huge variety of miRNAs have been identified in animals, plants, microorganisms and over 4000 miRNAs have been identified in humans (http://www.mirbase.org/), which is estimated to target more than 30% of the human genome that play important roles in cellular processes. Among their association to diseases like myocardial infarction, single point mutations, neurological and autoimmune diseases, their specific expression signatures in various cancer types have provided hope that miRNAs can be great candidates for early cancer diagnosis (Ardekani and Naeini, 2010). Regarding cancer, miRNAs possess two different characteristic feature as being either tumor suppressors or oncogenic miRNAs (Di Leva et al., 2014). First category comprises of miRNAs like miR-34, miR-let7, miR 143, miR 145, miR-133b, and miR- 126 whose expression is down regulated in cancer cells while oncogenic miRNAs are up regulated due to down regulation of tumor suppressor genes. miRNA-155, miRNA-21, miRNA-372, miRNA-373, miRNA-15a, miRNA-16-1, miRNA-34s, are some miRNAs that have been recently associated with various cancers (Negrini et al., 2007). Recent finding also shows the association between altered levels of certain circulating miRNAs with various diseases (Bostjancic et al., 2009a; Di Leva et al., 2014; Farazi et al., 2013; Islam et al., 2017; Montagner et al., 2014; Moreno-Moya et al., 2014; Piubelli et al., 2014; Rupaimoole and Slack, 2017; Virant-Klun et al., 2016; Weber et al., 2010; Yi et al., 2013; Zhuo et al., 2013).

1.4. miRNAs as therapeutics

It is now well known that aberrant miRNA expression is linked to cancer, and miRNAs have an important role in cancer occurrence, progression and metastasis. Therefore, tumor suppressors miRNAs or the antagomirs of oncomirs might be used as effective cancer therapeutics (Rupaimoole and Slack, 2017). The usage of miRNAs as therapeutics could be done in either of two strategies: for the purpose of either suppressing tumors via targeting oncomirs or replacement of tumor suppressor miRNAs 'miRNA replacement therapy'. Oncomir targeting could be done either direct strategies where oligonucleotides antimiRs, antagomirs or locked nucleic acids (LNAs) are used or indirect strategies based on virus-based constructs. For both the direct and indirect methods of oncomir targeting, the purpose is to block the expression of oncogenic miRNA by preventing its binding to RISC complex. These strategies could be shown to be effective also sensitizing tumors to therapy as in the example of tamoxifen where antagonizing miR-221/mir-222 may further sensitize cells to the drug (Miller et al., 2008). In the case of 'miRNA replacement therapy', tumor suppressor miRNAs could be replaced to restore loss function due to downregulation of certain miRNAs. For instance, due to decreased levels of miR 34a and let-7a in various cancers, several laboratories have been working on for the investigation of therapeutic benefit of "miRNA replacement therapy" (Miller et al., 2008; Wiggins et al., 2010).

Paradigm shifting genome editing tool, clustered regulatory interspaced short palindromic repeats (CRISPR)/cas9 system have been taken great attention in recent years. It has also been shown that, this technology can be applied to non-coding genes such as miRNA genes. It has been reported that, generation of knockouts for miRNAs such as miR-21, miR-29a could be achieved (Chang et al., 2016; Ho et al., 2015).

For further reading on therapeutic role of miRNAs reference paper should be followed (Kasinski and Slack, 2011).

1.5. miRNA analysis methods

Owing to their crucial roles in several diseases, especially in cancers, understanding the function and detection of expression levels of miRNAs is considered as very important non-invasive biomarkers and raised great attention not only by the academia but also by the industry where some miRNA based diagnostic tools/kits like mirVana, miRNAQrt-PCR kits,SmartFlare, etc. have already been launched by