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Review Article

miRNAs as biomarkers in human diseases

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Abstract

RNA interference (RNAi) is one of the primary machineries involved in the regulation of gene expression using small double-stranded RNA (dsRNA) in eukaryotic cells. MicroRNA (miRNA) is a class of small non-coding RNAs, regulating gene expression through canonical and non-canonical ways. Previous studies have shown that miRNA coding sequences make up 1% of the human genome and currently 1917 human miRNAs are displayed in the miRBase database. Expression levels of circulating miRNAs are related to various pathophysiological conditions such as cancer, infectious conditions, cardiovascular diseases, neurodegenerative diseases, and many more. Therefore, it is important to identify, detect and analyse miRNAs by using *in silico* and experimental analyses. In this review, after a brief description, we discuss the use of miRNAs for diagnosis and prognosis as biomarkers and biosensors in addition to miRNA-based therapies.

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1. Introduction

RNA interference (RNAi) is one of the primary machineries related to gene expression regulation in eukaryotic cells. RNAi delivered into cells initiate the degradation of messenger RNA (mRNA) via the cells' inner mechanism (Figure 1). This mechanism limits the gene expression by either suppressing transcription or activating a sequence-specific RNA degradation process (Almeida and Allshire, 2005; Deng et al., 2014; Xin et al., 2017).

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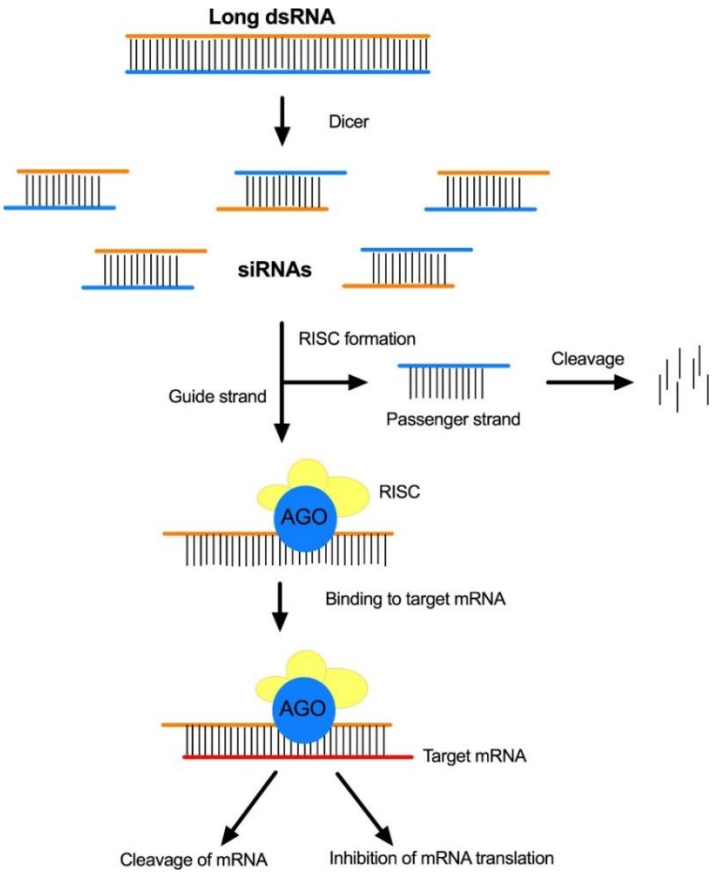

Figure 1. RNAi mechanism (Brüggenwirth and Martins, 2020)

In the process of gene expression regulation, Dicer protein binds to dsRNA, cleaving it into small pieces named as siRNA. These siRNAs bind to an Argonaute (Ago) protein which is part of the RNA-induced silencing complex (RISC). RISC divides siRNAs into two parts including passenger strand and guide strand. The passenger strand is degraded while the guide strand serves as a search probe that connects RISC for complementary RNA targets. After this identification, targeted gene expression could be regulated by various mechanisms (Brüggenwirth and Martins, 2020).

2. Small non-coding RNAs (sncRNAs)

sncRNAs with 20-25 nt in length including microRNA (miRNA), small interfering RNA (siRNA), and short hairpin RNA (shRNA) etc. have gained considerable attention in a wide range of applications in plant, animal and human (Liu and Paroo, 2010; Castel and Martienssen, 2013; Inal et al., 2014; Movahedi et al., 2018). These sncRNAs perform their mechanisms via both transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) (Axtell and Bowman, 2008; Covarrubias and Reyes, 2010). Both *in silico*

and experimental analyses have been performed to identify novel sncRNAs and their targets playing important roles in different metabolic pathways (Chetta et al., 2020; Zhang et al., 2020; El-Kafrawy, et al., 2021; Garcia et al., 2021; Rana et al., 2021). Obtaining data from these investigations provide new insight to figure out the complexity and functions of sncRNAs (Wittmann and Jäck, 2010).

2. miRNA

miRNAs are 17-25 nt in length, constituting up to 1% of the human genome (Friedman et al., 2009) and currently 1917 human miRNAs are displayed in the miRBase database (Kozomara et al., 2019). They regulate gene expression by binding to seed sequences of target mRNAs (Figure 2) (Bartel, 2004; Bartel and Chen, 2004). In eukaryotic organisms, miRNAs target mRNA involved in various metabolic pathways such as growth, development, abiotic and biotic stress (Eren et al., 2016; İlhan et al., 2016; Jian et al., 2017; Stepien et al., 2017).

Because of technological limitations to investigate miRNAs, the significance of miRNAs was understood after the discovery of lin-4 and let-7 which control nematode (*Caenorhabditis elegans*) development via incomplete base pairing to the 3′ UTRs of target mRNAs to prevent translation (Lee et al., 1993; Reinhart et al., 2000). For plants, Reinhart et al. (2002) detected miRNAs in a model organism, *Arabidopsis*. They revealed that ncRNAs could be arisen early in eukaryotic evolution (Reinhart et al., 2002). A single miRNA might potentially target several mRNAs whereas one mRNA might contain multiple binding sites for miRNAs (Chevillet et al., 2014).

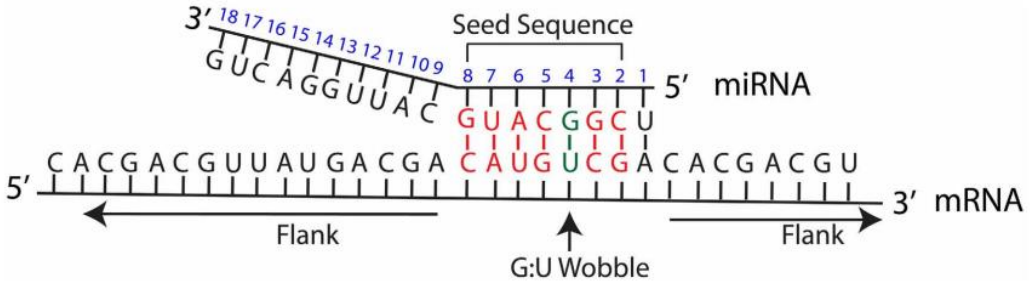

Figure 2. miRNA:target mRNA relationships. Blue numbers indicate miRNA position. The seed sequence is between 2-8 nt in miRNA position. Flank regions correspond to mRNA regions are found on either side of the seed region. Watson-Crick (WC) matches in the seed region are presented as red colour, and green colour for G:U wooble (Peterson et al., 2014)

3.1. miRNA Biogenesis

Biogenesis of miRNA starts with the processing of RNA pol II and III affecting primary miRNA (pri-miRNA) in the nucleus (Lee et al., 2004; Krol et al., 2010). Nearly half of the identified miRNAs are found in intragenic regions. They could be processed from introns and a few exons (de Rie et al., 2017). On the other hand, the remaining miRNAs are in intergenic regions regulated by their promotors (Kim and Kim, 2007). There are two different miRNA biogenesis classified as canonical and non-canonical pathways (Figure 3). miRNA-mediated silencing complex (miRISC) complex containing the sense miRNA strand which binds to the target mRNA through its 3′-untranslated regions (3 ′-UTR) in canonical pathways (Chevillet et al., 2014). On the other hand, about 60% of the relations between the miRISC complex and mRNA are non-canonical in human (Helwak et al., 2013) which means their chains aren't always entirely complementary to each other (Jonas and Izaurralde, 2015).

The production of pri-miRNA transcript is produced at the beginning of canonical RNA biogenesis and then microprocessor complex consisting of Drosha and DiGeorge Syndrome Critical Region 8 (DGCR8) cuts the pri-miRNA. As a result of cutting, precursor-miRNA (pre-miRNA) is produced and exported to the cytoplasm via Exportin5/RanGTP-dependent manner. In the cytoplasm, pre-miRNA duplex is bound to Ago family of proteins to form a miRISC. Another pathway, non-conical pathway is classified in Drosha/DGCR8-independent and Dicer-independent pathways. shRNAs are cut by microprocessor complex and exported to the cytoplasm via Exportin5/RanGTP similar to canonical pathway. Moreover, shRNAs were further processed via AGO2-dependent but Dicer-independent manner. Mirtrons and 7-methylguanine capped (m^7G)-pre-miRNA are processed to obtain mature miRNAs and this process is carried out by Dicer. Exportin5/RanGTP carries Mirtrons while Exportin1 is important for m^7G -pre-miRNA (Hayder et al., 2018).

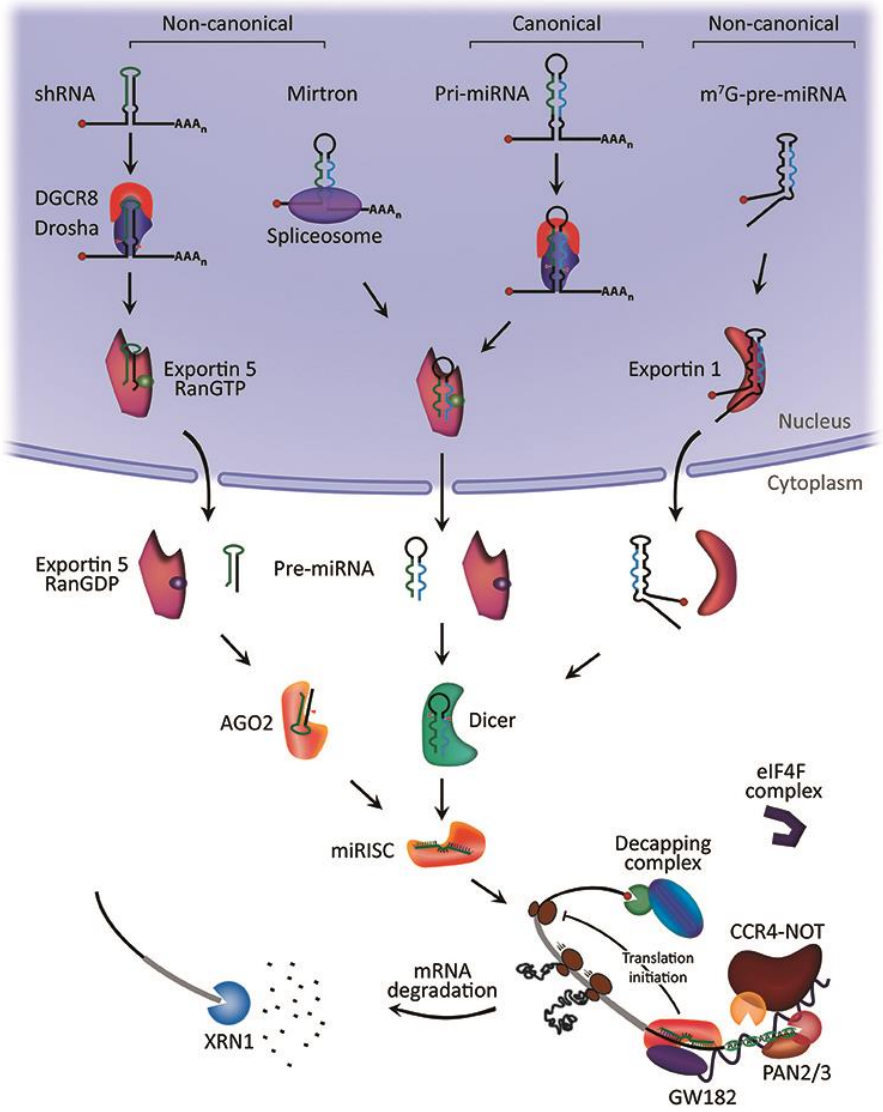

Figure 3. miRNA biogenesis (O'Brien et al., 2018)

3.2. miRNA Detection Methods

Novel miRNAs can be identified and analysed by using different methods. These methods are generally divided into groups *in silico* analyses and experimental analyses. Different miRNAs databases such as miRBase (Kozomara et al., 2019) miRandola (http://mirandola.iit.cnr.it/) and human infections (http://mir2disease.org/) etc. have been commonly used for detection of novel miRNAs related to disease and different metabolic pathways. By using reference sequences obtained from these databases, NCBI-BLAST, RNAfold, RNAHybrid, BLAST2GO and other related programs have been widely used for identification of miRNA and their targets via *in silico* analyses (Altschul et al., 1990; Kruger and Rehmsmeier, 2006; Conesa and Götz, 2008; Lorenz et al., 2011).

Northern blot analysis (Sempere et al., 2004; Válóczi et al., 2004), *in situ* hybridization (Kloosterman et al., 2006), real-time PCR (Chen et al., 2005; Wang et al., 2009), miRNA microarray (Thomson et al., 2004; Wang et al., 2014), and next-generation sequencing (NGS) (Wang et al., 2015) utilising massive parallel sequencing on platforms such as Illumina Genome Analyzer, ABI SOLiD, or Roche/454 Genome Sequencer FLX (Mardis, 2008) are employed in experimental analyses. Since each technology has strengths and weaknesses, Hong et al. (2021) proposed that miRNA-Seq for miRNA biomarker discovery and even identification of novel miRNAs. It is worth noting that miRNAs' expression profiles in plasma and serum have important for the potential usage of them as biomarkers for early disease diagnostics and the treatment of diseases (Nik and Shahidan, 2019).

3.3. Application of miRNAs as a Biomarker for Human Diseases

miRNAs are stable and tissue-specific molecules in extracellular compartment. These properties make circulating cell-free miRNAs as a promising class of non-invasive biomarkers for human (Hong et al., 2021). Circulating miRNAs are covered by membrane-bound vesicles such as exosomes. Numerous investigations showed that different pathophysiological conditions including cancer, liver damage, contagious conditions, cardiovascular diseases, neurodegenerative disease are related to expression of circulating miRNAs (Bhardwaj et al., 2013; Zeng et al., 2017; Aghili et al., 2018; Biswas et al., 2019; Coban et al., 2020; Teksoy et al., 2020).

3.3.1. miRNA-based biomarkers for diagnosis and prognosis

Circulating miRNAs might be used for diagnosis of infectious diseases such as Dengue, Ebola and others (Duy et al., 2016; Ouyang et al., 2016; Trilobet et al., 2020). Biswas et al. (2019) investigated 372 microRNAs in plasma samples from HIV-1 infected individuals to detect early/acute HIV-1 infection. They reported a miRNA panel (PeHIV-1) containing four differentially expressed miRNAs (miR-16-5p, miR-20b-5p, miR-195-5p, and miR-223-3p) in infected individuals when compared to healthy controls. Another frequent form of malignant disease, ovarian cancer, cause more than 150,000 women to die every year. On the other hand, this disease is diagnosed with late-stage disease. Therefore, it is important to improve early diagnosis biomarker systems. Berner et al. (2021) aimed to identify microRNAs to

detect ovarian cancer reporting that miR-15a and let-7a are highly suitable for ovarian cancer patients.

In addition to diagnosis, miRNAs have also been utilised as novel biomarkers in the prediction of prognosis (Zhang et al., 2019; Sun et al., 2019) and integrating multiple miRNAs might be more effective than single ones to predict prognosis (He et al., 2019). It is suggested that miR-103a-3p might be a potential non-invasive diagnostic and prognostic biomarker (Liu et al., 2022). Moreover, hsa-miR-584-5p detected as tumor suppressor miRNA is a potential candidate biomarker for coronary artery diseases (Coban et al. 2020).

3.3.2. miRNA-based biosensors

In recent years, biosensors with increased sensitivity via nanomaterials have gained more attention especially for early detection of diseases to prevent the progression. One of these investigations was performed by Aghili et al. (2018). They improved an electrochemical nanobiosensor based on the quantification of the circulating biomarker miR-195 for the early detection of Parkinson's disease. Specific and sensitive biomarkers are also very important for early cancer diagnosis. Zeng et al. (2017) demonstrated an ultrasensitive electrochemical biosensor, detecting microRNA biomarkers related to multiple pancreatic carcinomas. Four miRNAs, miRNA21, miRNA155, miRNA196a, and miRNA210, distinguished from healthy controls with very high sensitivity.

Exosomes found in human biological fluids have clinical importance in the diagnosis of various diseases. Song et al. (2021) examined the effect of the combination of exosomal miRNA-125b and miRNA-361 for the progression of Alzheimer's disease. They reported that sensor depending on these sequences can be applied clinically for AD diagnosis and has the potential to outstanding the field of dementia research and treatment in the future.

3.3.3. miRNA-based therapies

miRNA therapeutics in combination with chemotherapy could be used in the effective treatment of certain diseases. Apurinic/apyrimidinic endodeoxyribonuclease 1 (APEX1) is overexpressed in gastric cancer, performing several functions. Since miR-27a-5p inhibits this enzyme’s activity, He et al. (2021) studied with APEX1/miR-27a-5p axis, suggesting this axis

as a new therapeutic agent. Another investigation was performed by Zhang et al. (2020). They showed that the inhibitory role of miRNA-5119 mimic-engineered dendritic cell vaccines and also increasing role in anti-tumor immune response for mouse breast cancer model. In addition to miRNA/DC-based immunotherapy, platinum-based chemotherapy response depending on miRNA variants to detect the lung cancer susceptibility was also examined. Obtaining findings indicated that SNPs rs71428439 (miR-149), rs2910164 (R-146a), rs928508 (mir-30c-1) and rs629367 (let-7a-2) related with polymorphisms of rs11614913 (miR-196a-2) and rs9280508 (miR-30c-1) notably affected the patients' response, serving as potential clinical biomarkers to predict lung cancer risk (Fang et al., 2018).

5. Conclusion

In this review, we summarised the utilisation of miRNAs as biomarkers and biosensors, and even miRNA-based therapies for diagnosis and prognosis of diseases. Detection of miRNAs for a specific disease and also identification of differently expressed miRNAs between control and experimental groups provide new insight, especially for early detection. The results might be integrated into personalized medicine applications.

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