Original Article
The Role of Micro RNA and its Modulation in Diseases and Drug Response: The Spark in Bioscience
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\textbf{Abstract}

The cutting edge research area in the field of molecular biology is a micro-RNA study. It makes the milestone of gene regulation in plant and animal cell. Small noncoding RNAs rise as a micro-RNA and it reveals the fact of gene regulation with more information from \textit{Caenorhabditis elegans} to the humans from last two decades. In this review paper, we focus on the biogenesis micro-RNA and small-interfering RNA with the association of various proteins that efficiently take part in the gene silencing process as well as modulation of various micro-RNAs in disease conditions and under the drug stress causing the extent of drug efficacy by inhibiting the putative targeted messenger RNA.

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\textbf{Key words:} Micro RNA, short interfering RNA, Gene expression and Gene silencing

1. \textbf{Introduction}

MicroRNA study is an emerging spark area in the field of bioscience. MicroRNAs (miRNAs) are endogenous, around approximately 22 nucleotide small RNAs that are predominantly found in plants and animals and plays vital regulatory role by targeting putative mRNAs for cleavage leads to the translational repression or inhibition. About 1-3% of genome contributes for the miRNA transcription. The first miRNAs were characterized in the early 1990s, [1] but miRNAs were not recognized as a distinct class of biological regulators with conserved functions until the early 2000s. Since then, miRNA research has revealed multiple roles in negative regulation such as transcript degradation and sequestering as well as translational suppression. It also likely to be involve in positive regulation of transcriptional and translational activation of putative mRNA. There are different sets of expressed miRNAs are found in different cell types and tissues of organism [2]. Aberrant expression of miRNAs has been implicated in numerous disease states, and miRNA-based therapies are under investigation. Past few years have witnessed an explosive increase in the research reports on plant and animal miRNAs expression. So far, more than thousands of miRNAs have been identified experimentally and validated with cloning and computational approaches [4]. Functionality of the miRNA in gene silencing is proven as a novel system in organ development and it modulates to various stress responses such as oxidative, mineral-nutrient, mechanical stress as well as in drug stress involve in living system [3].

1.1 Discovery of MicroRNA

Discovery of miRNA has begun almost two decades ago during the larval developmental study of nematode worm \textit{Caenorhabditis elegans}. In 1993, Lee et al. [5] first time reported ~22 small nucleotide \textit{lin-4} RNA in \textit{C. elegans}, it was thought to be a nematode idiosyncrasy but further research proves the \textit{lin-4} miRNA take part in the repression of \textit{lin-14} mRNA translation by partial antisense complementarily to 3’ untranslated region (UTR) of \textit{lin-14} transcripts that encodes nuclear protein (LIN-14) which associated with the progression in developmental stages of larva of \textit{C. elegans}. In 2000, Ruvkun laboratory has reported second miRNA, let-7,
which represses 3'UTR of lin-14, lin-41, lin-28, lin-42, and daf-12 genes expression during developmental stages in *C. elegans* [6]. These miRNA could be derived from independent promoter, exonic, intronic and regulatory regions of genome [7, 25]. Most microRNA encoded from intergenic regions or anti-sense orientation to genes and it found to be conserved in many species [9]. About 16% of pri-miRNAs may be altered through nuclear RNA editing. Since after lin-4 and let-7 miRNA discovery, over thousands of miRNAs have been identified in viruses, worms, fungi, plants and primates through sequencing or computational predictions [10, 11].

### 1.2 Biogenesis of MicroRNA

MicroRNAs are ~22 nucleotide base fragment thought to be processed from longer endogenously transcripts with hairpin like structure [12]. Pre-miRNA is a precursor of mature miRNA derived from the Expressed Sequence Tags (ESTs), longer transcripts and polyadenylated mRNAs. One of the biggest breakthrough is that the siRNAs and miRNAs are functionally equivalent and miRNA can act as siRNA when it shows the extensive complementary to their target mRNA but extent of translational regulations are not clearly understood. Experimental evidences showed that the Polycistronic transcripts of 600 nucleotides pri-miRNA are also precursors of different miRNA like miR-23/27/24-2 and miR-17/18/19b-1 [13]. Pre-miRNA processed in the nucleus and upon maturation it exported to the cytoplasm where it associated with the nucleases and other proteins which help in recognition and making complementary with target mRNA, resulting in translation repression. The small interference RNA (siRNA) are believed to be an exogeneous double stranded RNA (dsRNA) molecule taking part in the cleavage of target mRNA. The detailed process of mi/siRNA biogenesis is mention below.

#### 1.2.1. microRNA biogenesis

The miRNA biogenesis pathway begins with the transcription of endogeneous miRNA gene by RNA Polymerase II. The nascent transcripts of 70-100 nucleotide long RNA form hairpin like structure flanking by sequence which is necessary for efficient processing. The hairpin structure of dsRNA recognised by nuclear protein complex consisting of dsRNA binding protein DGR8 and drosha enzyme called ‘ microprocessor’ which cut down of about 11 nucleotides from the two hairpin helix bases. The resulting hairpin structure is known as pre-miRNA. Pre-miRNA can also directly splice out from introns bypassing nucleases complex, are known as “mirtrons” found in *Drosophila, C. elegans* and mammals. Pre-miRNA hairpins are exported from the nucleus to cytoplasm through nucleocytoplasmic shuttle Exportin-5 (figure 1) which is Ran bound GTP dependant protein transporter.

Pre-miRNA are processed by Dicer (dark black oval shaped circle), possibly in conjugation with Argonaute proteins (dotted oval shaped black circle) which are of many and varies from organism to organism. There are seven and twenty four argonaute proteins are found in human and *C. elegans* respectively [15]. Such multi proteins complex binds to miRNA are called as miRgonaut. Dicer act as an endo-

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**Figure 1.** Diagrammatic representation of miRNA and siRNA synthesis and its interaction in gene silencing
Figure 2. Three different states of miRNA involved in pharmacogenetics event related to the drug efficacy. a) The cell with normal expression of the miRNA leads to the downregulation of the drugs inhibiting genes results into the increasing drug efficacy and sensitivity towards the abnormality in disease condition. b) The cell with normal expression of the miRNA but it fails in recognition of targeted genes because of single nucleotide polymorphism (SNP) in the 3'UTR leads to the increasing turnover of mRNA and its gene product shows the drug inhibition in the greater extend causing decreasing drug efficacy towards the disease and, c) miRNA expression can be disturb by deregulation at the level of expression, processing and maturation of miRNA leading to the less availability to the down regulated the targeted genes hence there is increasing in mRNA and its product causing inhibition of drug to the greater extent in disease conditions.

1.2.2 siRNA biogenesis
The second class of small RNA is termed ‘small interfering RNA’ (siRNA). It is believed to be generated from the exogenous nucleic acid such as from transgenes, transposons and heterochromatic repeats use as a substrates for RNA-dependent RNA polymerase and are converted to double-stranded RNA. Although there are no evidences of human cell generated endogeneous siRNA as well as enzymatic machinery used during maturation and target binding to putative miRNA. Experimental evidences shows that the Argonaute like protein binds to dsRNA and it takes part in formation of siRgonaute ribonucleoprotein complex (siRNP) and other related multisubunit nuclease complex that directs target RNA destruction [16]. Inhibition of transcript possesses different types of complementation -ribonuclease enzyme with RNase III activity and it interacts with the 3’end of dsRNA hairpin and cut away the loop joining the 3’ and 5’ arms, yielding an imperfect miRNA dimer complementation [14]. Mature strand of miRNA are associated with multi subunits of protein and it efficiently bind to the take part in the RNA-induced silencing complex (RISC) mediated gene silencing through ribonuclease activity [16].

1.3 MicroRNA and diseases
Nowadays, making and establishing the evolutionary relationship of miRNAs trying to decode the inhibition process involved in various diseases [14]. Confirmation towards the modulation of miRNA under various drug treatments and in disease conditions are being a promising research area and it runs extensively in research laboratory globally. Conclusive study on the miRNA put forward the information about its expression in the disease states. It has been suggested that the miRNA involve in protective mode during the abnormalities up to certain extent as well as it becomes more informative in the various disease conditions shown in Table 1. Neurological disorder like degeneration of neurons affects the expression level of neuronal miRNA. Experimental evidences in the C. elegan and the disease induced cell lines help to become informative about the
extent of impact of disease and the index of miRNA. For example, the lin-4 and let-7 mutant phenotypes observed in C. elegans can be interpreted as growth defect [20, 22] and it results into the abnormal progression in developmental stages. The let-7 family of microRNAs may also be regulators of the proto-oncogene \textit{RAS} and also showed the programmed cell death [21, 23]. Moreover, most of human miRNA are located at genomic regions which are linked to tumors [24, 25] as well as it enormously increases in chronic hepatitis C infected patient’s serum. The underlying mechanism of miRNA in disease conditions are still poorly understood, but it appears to be involving the inhibition of translational initiation [26]. In neuronal disease like in Alzheimer’s disease (AD), miR-107 expression levels decrease significantly it directly associated with the control by targeting beta amyloid precursor protein-cleaving enzyme 1 (BACE1), increasing BACE1 cleaves the myeloid precursor protein to generate neurotoxic-amyloid peptide which can be determined by miRNA profiling, affymetrix microarrays, bioinformatics predictions, and in situ hybridization. Thus, the deregulation of this protein may play a major role in the pathogenesis of AD.

Normally, miR-133b is expressed in the midbrain dopaminergic neurons (DNs). Abnormality in the dopaminergic neurons leads to the very low expression or absence of miR-133b causing the dysfunctioning of dopaminergic neurons such condition has trigger in the patients with Parkinson’s disease. The miR-133b regulates the maturation and function of midbrain DNs through down regulation of expression of the pair-like homeodomain transcription factor Pitx3. Furthermore, studies in mammals and in invertebrates have suggested that miRNAs are involved in neuroprotection and schizophrenia. Successive research indicated that the neurodegenerative diseases might
because of the alteration of different cellular pathways. Care et al. described the role of mir-133 targeting RhoA factor, a GDP-GTP exchange protein that regulated cardiac hypertrophy [27]. Different modulation of same miRNA cause different types of abnormality in living organisms, miR-1 is over expressed in individuals with coronary artery disease and when over expressed in normal or infracted rat hearts, it exacerbates arrhythmogenesis by silencing the ion channel genes. Knockdown of endogenous miR-1 can inhibit ischemic arrhythmias, which might represent a new approach for anti arrhythmic therapy. Recently Lovis et al. explored two miRNAs, miR-124a and miR-96, which modulate the expression of different proteins involved in insulin exocytosis and in diabetes condition [28] while, Poy et al. has suggested that miR-375 also acts in the regulation of insulin secretion by islet cells of pancreas [29].

1.4 MicroRNA and drug response
Micro RNA involvement in drug targeting is a challenging research area in the field of molecular medicine. It has been noted by molecular biologist that a new approach of the pharmacogenomics and the miRNA profiling to the drug response would provide the information about the miRNA expression and gene regulation. Micro RNA is a master regulatory element that controls the regulation of most of the genes by binding to the 3' UTR of mRNA result into inhibition of translation of the genes. If the complementary between a mi/siRNA and the targeted transcript is partial, then translation of the target mRNA is repressed, whereas if the complementary is extensive, the target RNA is destabilized by endonucleolytic cleavage [30]. Influence and availability of the drug to tissue, various cellular drugs modulating enzymes get expressed but the expressions of the genes are guarded by the miRNA interference results into translation inhibition and reduction in the efficacy of the drugs. A different scenario of miRNA in figure 2 shows how the miRNA affect the drug efficacy in disease conditions. For example NAD(P)H dehydrogenase, quinone1 (NQO 1), vitamin D receptor (VDR) etc., plays a novel role during drug intake and in the disease condition such as cardiovascular diseases [31], diabetes [29] and neurological disorders [46]. While in the cancer, miRNA are differentially deregulated by the individual type of cancers and hence it can be used as a potential diagnostic tool for the identification of the type of cancers and the treatment purpose [32, 33]. Likewise, miRNA expression variation determined from healthy to disease tissues provides the information about the potential expressed miRNA in presence of different drugs. Under the influence of the drug administration to the cell lines or patient, different types of tissue specific genes get expressed and it can be regulated by the miRNA at the translational level. In most of the in vitro and in vivo experiments miRNA downregulated the genes by expressing in greater number but it also found that the upregulated other genes results in the decrease of specific miRNA. Besides the central role of miRNA like miR21 is often upregulated and has oncogenic properties [33]. Efficacy of the drugs are depends upon the expression of the genes including cytoplasmic as well as trans-membrane transporter proteins allows enhancing of drugs activity towards the target. Such modulation of miRNA under the different drug exposure (see Table 2) plays a novel role in the regulation of putative genes and hence it can be used as a gene regulator and pharmacogenomic marker. Invitro study in Pancreatic ductal adenocarcinoma cell lines contributes to the upregulation of the miR21 and posses gemcitabine drug resistance [34] while, tumor specimens analysed form the 21 pancreatic patients were found remarkably increased 1000 folds of mir21 comparable with the normal cell [35]. The role of miR21 in gemcitabine resistance exemplifies about how the drastic changed often seen in miRNA expression during tumorigenesis and the potential of targeted drugs towards miR expression level. Murakami et al [36] determined the responsive miRNA which showed the resistance to interferon in the chronic hepatitis C patients confirm by the hepatitis C virus in the individual patient serum. Predominant role of bioconversion enzyme such as in liver enzymes belongs to large family of Cytochrome p 450 that take part in the conversion of complex compound to the simpler bioactive product and it also associated with the control mechanism of miRNA. A cascade of the cell signaling promotes the expression of CYP subfamily members for enzymatic reaction towards the drug or complex molecule conversion. Anticoagulant drug like warfarine shows the adverse drug events during the metabolism by CYP1A1 and it targeted by miR125b. The experimental confirmation of miR125b shows the association with the optimal dose of warfarine while, the same miR-125b showed the calcitriol resistance by affecting the target protein level mentioned in Table 2 [37, 38]. From the listed examples, similar miRNA showed the resistance towards the multiple drugs and hence it plays a decisive role in the induction of drug resistance and clues about the mechanism for the targeted drug. This information could help in designing of more effective treatment protocols for the cancer diseases via cocktail of combinational cancer drugs acting through different pathways so that the drug resistance will be minimized.

1.5 Conclusive remark
The spark of miRNA is a novel bioscience research area that could help to understand the miRNA mediated molecular mechanism of cell regulations. Experimental validations open new strategies of the miRNA interactions and gene silencing during the stress conditions that gives us clues to make the progression in the drug targeting via control mechanism of miRNA regulation in the targeted tissue. From the various literatures it can be concluded that, through the miRNA profiling one can increase the efficacy of the drugs by administrating the combinational drug therapy which makes a
synergistic effect on the targeted tissues results into lowering of drug inhibitions. In future, major susceptibility of cancer patients can be minimized through the miRNA regulation by lowering drug toxicity and resistivity. Hence, with the use of high throughput technology and computational tool like nanotechnology and bio-molecule design, we can certainly achieve the promising challenges in the area of miRNA research.

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