Voyager: Voll. V, Dec. 2014, 34-41: 2014
ISSN :0976-7436 : INDEXED AND ABSTRACTED

A REVIEW ON MICRORNA AND ITS DISCOVERY

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Introduction

MicroRNA is small, non-coding RNA composed of 18-24 nucleotides. They act as negative regulators of gene expression at post transcriptional level. MicroRNA binds to mRNA at its complementary site and prevents the synthesis of protein. In 1993,miRNAs were discovered from nematode Caenorhabditiselegans and Lee et al [1]found lin-4, an important gene for post embryonic development of C.*elegans*, but does not code for protein. Since then, this small RNA molecule plays an important role in regulation of gene expression. miRNAs play an important role in different biological process such as cell proliferation, cell growth and organization, as well as apoptosis, biogenesis, transcription, cell cycle and fat metabolism. Till date around 3% of genes in human has been found to encode miRNAs and upto 40-90% of human encoding genes are under miRNAs mediated gene regulation [2]. Any deregulation in miRNA biogenesis or its function may lead to various diseases in human as the target genes of miRNA are involved in several metabolic disorders such as various forms of cancers, heart diseases, neurodege - nerative disorders, diabetes etc. With recent experimental and computational basis, it has been indicated that single miRNA can regulate expression of several genes and expression of single gene can be controlled by several miRNAs [3].

Biogensis Of miRNA

Like mRNA, miRNAs are transcribed from DNA by RNA polymerase (II) or (III) which is the first step in the biogenesis of miRNAs. In this step pri-miRNA is formed inside the nucleus from miRNA gene[4]. The pri-miRNA is capped, polyadenylated and then processed by RNAase III endonuclease Drosha and its cofactor DGCR8 (Di George Syndirome Critical Region Gene 8). These complexes of proteins are known as microprocessor

complex which trim the pri-miRNA to produce shorter hairpin of around 70 nucleotide in length as pre-miRNA (precursor-miRNA) with 2 nucleotide overhang at 3'end. The pre-miRNA is then transported to the cytoplasm by Ras related nuclear protein-guanosine 5'triphosphate (RAN-GTP) dependent export receptor Exportin-5.

This pre-miRNA is stabilized through their interaction with EXP-5 [5]. The transported pre-miRNA typically comprise of stem of ~22bp, a terminal loop and a 3' overhang of~2 nucleotides [6]. This 3' overhang of ~2 nucleotide indicate the cleavage by a second RNAase III endonuclease, a cytoplasmic Dicer. This lead to the formation of ds miRNA (double stranded miRNA) of 22 nucleotide in length. This ds-miRNA is assembled with RISC (RNA INDUCED SILENCING COMPLEX). These are mediated by the RISC leading complex (RLC). Argonuate proteins are active RNase enzyme and extremely conserved in the RISC. This mature miRNA is now capable of regulating its target mRNA. Any deregulation of this processing results in disturbance in miRNAs genesis and lead to oncogenic consequences.

Target Base Pairing Of miRNAs

miRNAs binds to their cognate mRNAs by complementary base pairing to its multiple sites in 3' Untranslated region (UTRs) and that involves Watson Crick A:U and G:C pairs but also the G:U pair, the 5'end of miRNAs consisting of 2 to 8 nucleotides is called as seed region [7]. The miRNAs binding sequence in target is referred to as the miRNAsRecognization Element or MRE and only seed region is complementary to this MRE rather than full length of miRNAs. In general, most of targeted mRNAs had interaction with miRNAs through their 3' UTRs [8]. Destruction of mRNA by cleavage of its phosphodiester bonds or inhibition of its expression are based on the degree of complimentary[9]. Currently, various computational tools are used for predicting the targets of miRNAs such as Target Scan (http://www.targetscan.org), EMBL (http://russel. emblheidelberg.de) and miRBase(http://microrna. sangerac.uk).

Mechanism Of Gene Regulation

Evidences have shown multiple methods of miRNA-mediated gene regulation which includes translational inhibitor, increased mRNA deadenylation and degradation or mRNA sequestration. However, the interlation in these diverse mechanisms are yet not clear.

MiRNAs mediated mRNA degradation requiresArgonaute proteins, Processing body (P body) which consist of GW182, deadenylase complex (CCR4, NOT1, CAF1),

decapping enzyme Dcp2 and several decapping activators including Dcp1, Ge-1, EDC3 and RCK/p54 [10].

Evidences have reported that miRNAs may inhibit mRNA translation at initiation steps. Ago 2 protein associates with both eIF6 (elongation factor) and large ribosomal subunits and consequently prevents them joining with small ribosomal subunits. Thus, large and small ribosomal subunit are unable to associate as 80s ribosomal complex and thus prevents translation. miRNAs also prevents initiation of translation by inhibiting eIFE4E/cap and poly(A) tail function and repressed complexes moves to P bodies for mRNA storage or degradation [11].

miRNAs binding to mRNA at its 3'UTR induces deadenylation and this is followed by decapping of target mRNAs. The deadenylation of Mrna is by deadenylasecompex (CAF-1, CCR-4,NOT-1) and mRNAdecapping by decapping complex (DCp1 and Dcp2) and further exonucleolytic degradation by exonucleaseXrn 1 [12]. GW182 which is a component of P body recruits miRNAs targets through direct interaction with Ago proteins and thus contributes to translational repression. Some evidences have also shown that in translational process polyribosomes were actively involved but nascent polypeptide chain could not be deected by immunoprecipitation due to protein degradation.

Roles Of miRNAs In Disease

i. miRNAs IN Neurodegenerative Diseases

Neurodegenerative diseases are group of neurological disorders that results due to deterioration of neurons which leads to disabilities and possibility of death. Environmental and genetic factors seem to be major factors of neurodegenerative diseases and aging has been found to be common risk factor. Different forms of neurodegenerative diseases are recognized but the lines that separate them are often unclear. Symptoms such as motor impairment and memory loss occur in many different types of neurodegenerative diseases such as Alzheimer's, Parkinson's, prion and polyglutamine disorder including Huntington's disease are well known neurodegenerative disorders [13].

2. miRNAs IN Alzheimer's Disease

Alzheimer's disease(AD) is best known degenerative disease that affects the central nervous system. AD is chronic disease characterized by early memory loss followed by other cognitive defects: aphasia(long disturbances), agnosia (failure to recognize people), apraxia (inability to perform motor acts). Studies has suggested that dysregulation in miRNA expression could be associated to aging and contribute to

AD. AD is the most common cause of dementia in aged population. About 1% early onsetf familial form of disease (onset before 60 to 65 years) is due to mutation in three genes, APP, Presenilin 1 (PSEN 1) and Presenilin 2 (PSEN2)which cause overproduction of Aâ and â amyloid cleavage enzyme (BACE 1) which lead to processing of APP and further initiation of Aâ production. The molecular mechanism involving miRNAs and expression of BACE1 and APP are emerging in AD. By using microarray and insitu hybridization of superior medical frontal cortex of AD, It has been found by Nelson's laboratory that the expression of miR-107 decreased during progression of disease but BACE1 mRNA increases. Similarly mouse brain development from E17 to 1 year, BACE 1 protein level decrease was correlated to miR-29a/b-1 upregulation while BACE1 mRNAlevel was stable [14]. Interestingly, two other miRNAs that is miR-298 and miR-328 regulates BACE1 protein expression in cultured neuronal cells [15]but as the work was done on transgenic mice, additional work needed to determine whether ALL these miRNAs are really active in human brain.

It has been shown that AD can be caused by increased expression of the APP gene and 3'UTR of APP mRNAis a potential target for several miRNAs. Recently, experimental approach using human HEK-293 cells demonstrated that miR-106a and miR-50c negatively regulate expression of reporter genes containing their predicted target sequence present in the APP 3'UTR [16].

It has been found from sporadic AD patients that miR-106a along with let 7, 101, 15a and 106b were found to be downregulated in anterior temporal cortex of AD patients [17]. Thus, with the referred evidences it can be concluded that up and down regulation of miRNA leads to neurodegenerative diseases in humans.

3. miRNAs In Heart Disease

It is becoming apparent that aberrant expression of miRNAs is related to a variety of disease states like cancer, diabetes and heart failure. Stress induced upregulation of miRNAs can lead to downregulation of a set of targeted mRNAs whereas downregulation of miRNAs can result in upregulation of target mRNAs because of the loss of inhibitory control miRNAs on its target mRNA.

The adult heart responds to an injury or hemodynamic overload by activating a variety of intracellular signaling pathways that promote myocyte hypertrophy, remodeling of extracellular matrix that further leads to cardiac arrhythmias and failure [18]. These studies have revealed a pattern

of upregulation and downregulation of miRNAs such as miR-1, miR-29, miR-30,miR-133 and miR-150 have often been found to be downregulated and miR-21, miR-23a, miR-125, miR-195, miR-199 and miR-214 are upregulated with hypertrophy.

Cardiomyocyte Hypertrophy And Remodeling

Cardiomyocyte hypertrophy is the dominant cellular response to all forms of hemodynamic overload, endocrine disorders and myocardial injury. This might further result in loss of cardiac function and heart failure that corresponds to sudden death. In vitro experiments using either overexpression and knockdown of miRNAs in cultured cardiomyocytes indicate that there are several miRNAS involved in cardiomyocytes hypertrophy [19].

MiRNA-21 is one of the miRNA that is consistently induced by cardiac stress and act as regulator of cardiac growth and fetal gene activation in primary cardiomyocytes in vitro. Its role in myocyte hypertrophy was demonstrated by Chang et al. This reported that knock down of this miRNA can supress cardiomyocuyte growth and fetal gene expression in response to hypertrophic agonists. Sayed et al has reported that miR-21 modulates the formation of cellular protrusions through regulation of sprout 2, an intracellular inhibitor of Mitogen activated protein kinase signalling [20].

Another miRNAs that is upregulated in hypertrophic hearts is miR-195. The formed expression of miR-195 in primary cardiomyocytes or in the hearts of transgenic mice will drive to hypertrophic growth and myocyte disarray that result in cardiomyopathy and heart failure [21]. miR-195 belongs to the miR-15 family which consist of miR-15,16,195,424,497. Both miR-15a and 16-1 act as negative regulator of BCL2 that induces apoptosis in cancer cells [22].

miR-1 is a muscle specific miRNAs that forms a bicistronic cluster with miR-133 [23]. Both miRNAs are expressed at low levels in humans and mouse models of cardiachypertrophy and their overexpression can inhibit cardiac hypertrophy.

miRNAs And Carcinoggenesis

Recent research indicates that alterations in the expression of several miRNAs are often present in human cancers, suggesting roles of miRNAs in carcinogenic processes. In 2002, Calin et al [24] correlated aberrant miRNAs expression with cancer. Few miRNAs such as let-7, miR-15a/miR-16-1 cluster and neighbouring miR-143/miR-145 [25] have been reported to be reduced in some malignancies suggesting their potential tumor suppressor activities. In contrast, some other

miRNAs such as miR-17-92 cluster and miR-155/BIC are known to be overexpressed suggesting their oncogenic potentials [26].

Discovery and research on microRNA has revealed that these small non-coding RNA not only regulate expression during normal development but plays an active role in several diseases. Recently, miRNAs have been found that regulate pulmonary vascular homeostasis and could serve to treat pulmonary arterial hypertension. With going on research work and understanding about the molecular mechanism of miRNAs, researchers have evolved the role of miRNAs in pathophysiology of depression and contribution to the action of antidepressants. Thus, with further research about microRNA, there will be a notable change in persistence of diseases.

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