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ROLE OF MICRO RNAS ON ENDOTHELIAL CELLS: POTENTIAL BIOMARKERS OF CARDIOVASCULAR DISEASE-A MOLECULAR PERSPECTIVE

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Abstract: Endothelial dysfunction is an early predictor and primary biochemical change of a coronary artery disease. Several circulating micro RNAs (miRNAs) associated with development of atherosclerosis have been studied as potential biomarkers of cardiovascular disease. Aim of the present review was to study the role of miRNAs on endothelial dysfunction and their association with CAD. Cell-specific effects of miRNAs revealed that these can modulate angiogenesis of endothelial cells by targeting the stem cell factor receptor causes cardiac dysfunction. The present review concluded that circulating the panel of miRNAs plays as potential role on early prediction of endothelial dysfunction associated with CVD. Early prediction was useful to revert of endothelial dysfunction in initial phases of atherosclerosis. These circulating miRNAs can be used as early biomarkers of endothelial dysfunction and showed association with nitric oxide bioavailability.

Key Words: Micro RNAs, Endothelial dysfunction, Atherosclerosis, Biomarkers

I. Introduction

MicroRNAs are an abundant class of highly conserved single-stranded non-coding endogenous RNAs of ~22 nucleotides in length, which negatively regulate expression of genes at the post-transcriptional level by inhibiting translation of protein from the messenger (mRNA) or promoting its degradation. miRNAs play an important role in forming the transcriptomes and proteomes of eukaryotic organisms. Total number of miRNAs is unknown but it was estimated that the human genome encodes at least 800 miRNAs. Most of the genes of miRNA located in the introns of protein-coding genes controlling 30% of protein-coding genes. [1]

History of microRNAs

The first miRNA to be discovered was lin-4 identified in 1993 during screening for defects in the temporal control of post-embryonic development in *Caenorhabditis elegans*. The second miRNA let-7 was also discovered in C.elegans in the year 2000. Let-7 encoded a 21 nucleotide RNA which controls the L4 to adult transition of larval development. Trouncing of let-7 activity causes reappearance of larval cell fates during adult stage of development. It has been detected Let-7 activity in vertebrates, ascidian, hemichordate, mollusk, annelid and arthropods. Let-7 expression has also been shown in human tissues including brain, heart, kidney, lung, liver, stomach, small intestine, and thymus. MiRNAs are named using the prefix "miR" and a unique identification number, e.g., miR1, miR- 16 etc. Mature miRNAs differing only in one or two positions are given suffixes (miR 10a and miR 10b). The mature sequences are designed as "miR" in the database, where as the precursor hairpins are labeled as "mir". [2-5]

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

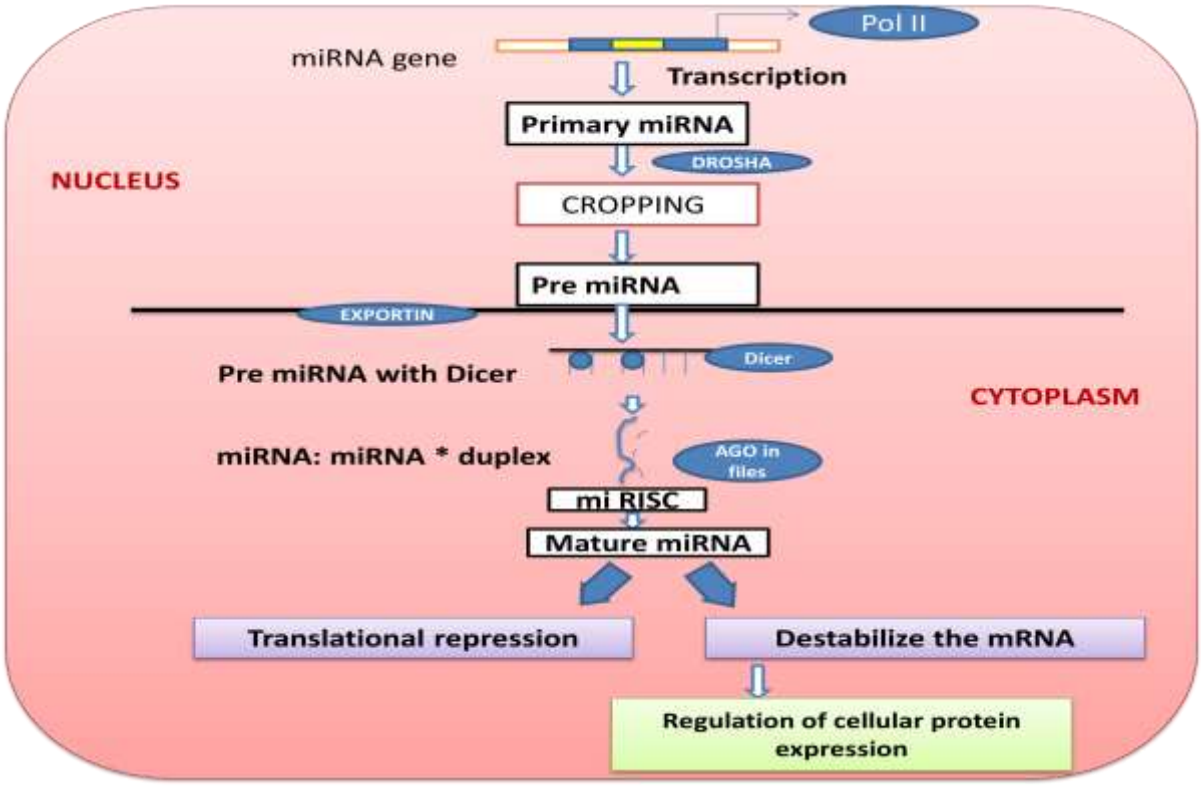
Biological Role of miRNAs

miRNAs have been involved in post-transcriptional regulation of gene expression. miRNAs play a pivotal role in the cell cycle, immune modulation, metabolic processes, and stem cell differentiation. Various miRNA expressions have been observed in numerous diseases. It has been extensively studied as biomarkers and therapeutic targets. Several circulating miRNAs were associated with atherosclerosis development have been studied as potential cardiovascular disease (CVD) biomarkers. [6]

Micro RNA Biogenesis:

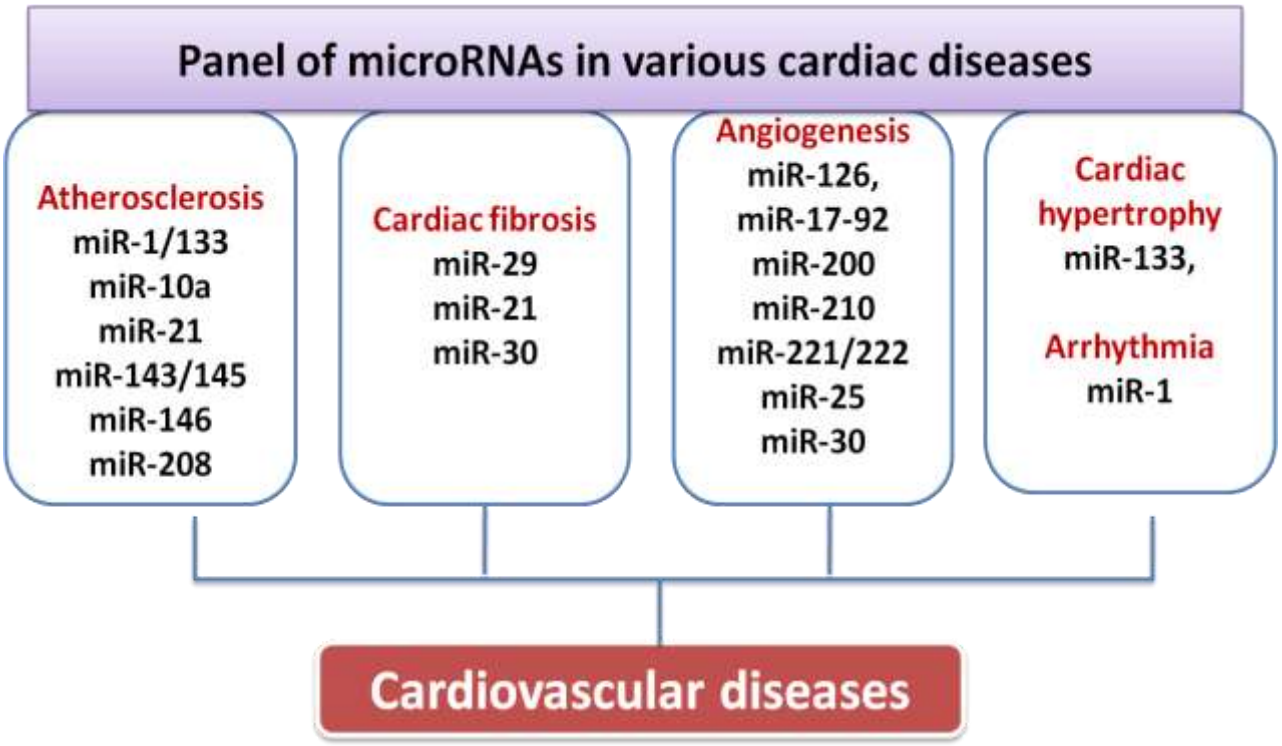
The miRNA transcription depends on the host gene. Consequently, in the nucleus of mammalian cells, most of miRNAs genes are transcribed by RNA Polymerase II (RNA Pol II) producing the long primary miRNAs (pri-miRNAs), 500–3000 base pair molecules. Later folding of pri-miRNA specific regions into hairpin structures is a key aspect of the initial pri-miRNA processing. In sequence, long pri-miRNA transcripts are cleaved by a microprocessor multi protein complex containing RNA-binding proteins such as DGCR8 and DROSHA thus, generating pre-miRNAs, the miRNA precursor form. Pre-miRNAs are hairpin structures that are formed by this cleavage. Then, pre-miRNAs are transported to the cytoplasm by exportin-5 protein, where cytoplasmic RNase III Dicer-1 subsequently cleaves them into unstable mature asymmetric duplex miRNAs. One strand of the duplex, usually with relatively lesser stability of base-pairing at the 5-end, is intended to become the mature guide miRNA. Its undergo phosphorylation at 5-end is essential for the interaction with an Argonaute (AGO) proteins. The other strand was referred as passenger strand. The two strands undergo further processing mediated by specific proteins. Thus, in the cytoplasm, selectively associated with AGO proteins, the guide strand of miRNA duplex is getting incorporated into the RNA-induced silencing complex (RISC) that is facilitated by Dicer-1/trans activation-responsive RNA-binding protein (TRBP), as well as other proteins like PACT protein, the activator of interferon-induced protein kinase R. The passenger strand is either degraded by linked to RISC AGO proteins/performs important regulatory functions for the guide strand. Previous supportive literature reports on the functional activity of passenger strands. The RISC integrated active single-stranded miRNA represses mRNA translation, i.e., destabilizes mRNA transcripts by cleavage or de adenylation, thus, regulates protein expression. On the whole, despite the limited knowledge about the upstream mechanisms controlling miRNA abundance, plentiful studies confirmed that miRNA expression is controlled by Dicer and DROSHA processing complex. [1-6]

Figure 1: Biogenesis of miRNAs



ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

Figure 2: microRNAs panel in various cardiovascular diseases



Role of micro RNAs in CVD:

A number of miRNAs including miR-1, miR-16, miR-27b, miR-30d, miR-126, miR-133, miR-143, miR-208 and the let-7 family are expressed in healthy cardiac tissue and play a role in normal cardiac maintenance as well as in diseases of the heart.30 The miR-143/-145 (miR-143/-145) which are profusely expressed in vascular smooth muscle cells (VSMCs) have been shown to be involved in the differentiation and determine the VSMC phenotypic switching. They could thus serve as potential drug targets for atherosclerosis, hypertension, and CAD. Other miRNAs concerned in causing CVD include miR-1 in cardiac arrhythmia, miR-29 in cardiac fibrosis, miR126 in angiogenesis and miR-133 in cardiac hypertrophy. The miR-1 has been shown to have a role in morphogenesis of cardiac cell, controlling cell fate of different lineages and thus helping in normal development of cardiac chambers. miR-1 has been reported to be over expressed in individuals with CAD, It thus represents a potential drug target in patients with arrhythmias (Figure 2).

Circulating micro RNAs as early predictors of atherosclerosis:

It is well known that endothelial dysfunction is early predictor of atherosclerotic cardiovascular disease. Endothelium is among the first line of the body's defence system. During inflammatory process, the Endothelial cells (ECs) turns to be activated. The ECs of artery activation induces increased vascular permeability for plasma proteins, the expression of proinflammatory cytokines, chemokines and enzymes, and an up regulation of cellular adhesion molecules. Nuclear transcription factor-κB (NF-κB) regulates the expression of adhesion molecules, such as intercellular adhesion molecule1 (ICAM-1), vascular cell adhesion molecule-1, and E-selectin that play a pivotal role in leukocyte-endothelium interactions. Chemokines, such as monocyte chemo attractant protein 1 and IL-8, are initiated through activation of the classical pathway of NF-κB. [6, 7]

Role of miRNAs on Endothelial Cell Function:

It is well known that the exposure of the endothelium to various stimuli, such as hypoxia, proinflammatory cytokines, oxidative stress, hypertension, hyperglycemia, shear stress, ageing, or injury, compromises the function of endothelial cells resulting in their increased proliferation, migration, apoptosis, senescence, angiogenesis, and inflammation. Previous studies demonstrated that specific classes of miRNAs involved in the control of pathways regulating function of ECs, mainly maintenance of vascular integrity, proliferation, and migration of ECs. [8, 9]

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

Zhang X et al,. reported that miR-221 can be dys regulated in endothelial progenitor cells (EPCs) and involved in the regulation of their function. They found that miR-221's EPC expression levels were significantly elevated in subjects CAD with atherosclerosis, compared to healthy volunteers, and also it was noted that up regulated expression of miR-221 diminished the proliferative ability of EPCs. [10] Additional studies in vitro analysis have been proposed by various researchers found that cell-specific effects of miR-221 and miR-222 revealed that these miRNAs can modulate angiogenesis of ECs of vasculature by targeting the stem cell factor receptor c-kit and also, indirectly regulate the expression of endothelial nitric oxide synthase (eNOS). [11-15]

Role of miRNAs on Endothelial dysfunction:

Nitric oxide is a key regulator of growth and migration of ECs of vasculature, remodelling, and angiogenesis. Its impaired bioavailability is a hallmark of endothelial dysfunction in patients with atherosclerosis. [16-19] Bonauer A et al., opined that the endothelial cell-restricted miR-126, by reducing the expression of sprout-related protein 1, can promote developmental angiogenesis in vivo, consequently, is deeply involved in the aid of endothelial dysfunction. Whereas, the over expression of miR-92a can block angiogenesis and reduce migration of ECs in vitro and in vivo. In addition to that, by targeting vascular endothelial growth factor receptor 2 (VEGFR2) and fibroblast growth factor receptor 1 (FGFR1), miR-129-1 and miR-133 were able to suppress key factors of angiogenesis in vitro. It was reported that the functional role of miRNAs such as miR-146a, miR-147, miR-126, and miR-155 among others in vascular remodelling response to the development of plaque, an essential component of atherosclerotic disease. [20-22]

Association of micro RNAs with share stress:

In ECs, regulation of specific miRNAs by shear stress can promote either vasculo protective or proatherogenic effects. The down regulation of miR-92a by shear stress increased eNOS expression, whereas, the up regulation of miR-19a contributed to the shear stress-induced cellular proliferation inhibition. However, the low shear stress-induced expression of miR-21 lead to pro-inflammatory phenotype of ECs. Although, in response to prolonged unidirectional shear stress, up regulated miR-21 displayed atheroprotective function by reducing apoptosis and increasing nitric oxide availability. [23, 24]

Role of micro RNAs on control of redox balance:

miRNAs are directly involved in the control of the redox balance in ECs of vasculature. Along with mentioned above eNOS, NADPH oxidase, superoxide dismutase, glutathione peroxidase, and thioredoxin-dependent peroxidase (TrxR1) are crucial enzymes for the maintenance of redox balance in cells. miRNAs can regulate the function of NOX subunits. Varga ZV et al., opined that hypercholesterolemia induced miR-25 inhibition caused a significant increase in NOX4 expression in the heart leading to cardiac oxidative stress and finally leads to cardiac dysfunction (Varga, Z.V 2013). [25] Three miRNAs, such as miR-106b, miR-148b, and miR-204, by direct targeting of NOX2, this activation is the major source of reactive oxygen species in endothelial cells. [26] Furthermore, directly targeting SOD2 and SOD3, miR-21 displayed pro-oxidative effects. [27, 28] Under oxidative stress conditions, miR-125a alleviated miR-125a-mediated translational repression of TrxR1 was down regulated, which thereby, functioned as antioxidant defence control. [29] miR-133 over expression that increases the activity of GPx which leads to protects endothelial cells from oxidative stress-induced apoptosis. [30] Furthermore, miR-148a is expected to be involved in the oxidative stress-determined reduction of NO bioavailability. It can reduce eNOS activity which contributes to early atherosclerotic lesion formation. [31]

Role of miRNAs on Endothelial Cell Senescence

miRNAs, such as miR-134a, miR-217, miR-30, and miR-146a have vital roles in the endothelial ageing. Vascular ageing is closely involved in alterations in the biomechanical and structural properties of the ECS and VSMCs, thus, endothelial dysfunction, as well as enhances arterial stiffness. [32] Predominantly, endothelial cell senescence is important in atherosclerosis. During ageing process, progressively expressed in endothelial cells miR-134a and miR-217 promoted endothelial cell senescence via suppression of silent information regulator 1 (SIRT1), the key regulator of longevity and endothelial function. [33-35] On the other hand, miR-146a can delay endothelial cell senescence that plays key role to protect vasculature during ischemic or inflammatory stress conditions. [36] Interestingly, targeting the same genes, different miRNAs can produce opposite effects, and this should be considered while analyzing the role of miRNAs. For instance, mentioned above miR134a, by down regulating SIRT1, was able to promote endothelial cell senescence, whereas, let-7g produced the inhibitory effect on the senescence of ECs through targeting the same SIRT1 gene. [36]

ISSN- 2394-5125 VOL 7, ISSUE 15, 2020

Role of miRNAs in Regulation of Endothelial Cell Apoptosis

Apoptosis of ECs plays a vital role in the initiation, progression and development of the atherosclerotic lesion. The apoptosis of ECs is responsible for plaque instability since it can predispose to arterial thrombosis that can lead to acute coronary occlusion and sudden death. [37] Abundance of evidence suggests that several miRNAs are implicated in regulatory mechanisms of ECs apoptosis. Some have anti-apoptotic effects, while others are pro-apoptotic. Chen L et al., demonstrated that apart from facilitating angiogenesis, the most endothelial cell abundant miR-126 can inhibit apoptosis of vascular ECs. [38] MiR-495 significantly promoted HUVEC proliferation by targeting chemokine (C-C motif) ligand 2 (CCL2) and inhibited apoptosis by affecting the cleaved caspase-3 expression. [39] On the contrary miR-132 repressed proliferation, capability, and migration of tumor necrosis factor alpha (TNF-alpha)-induced HUVECs promoting apoptosis. In addition, endothelial apoptosis, likely contributing to the loss of ECs, may expose surface extracellular matrix potentially stimulating platelet adherence and aggregation and the subsequent thrombus formation on the surface of the unstable plaque. In this aspect, it was established that promoting ECs apoptosis platelet-secreted miR-223, by targeting the insulin-like growth factor 1 (IGF-1) receptor, can participate in the formation of thrombus occurring in the later stage of atherosclerosis. [40] It was observed that during the formation of atherosclerotic plaque, apart from the regulation of endothelial apoptosis, miR-223 can also participate in the development of the inflammatory response by regulating neutrophil function. [41] In addition, Zhang T el al opined that a new role of miR-30 mediating translational control of autophagy-related gene 6 (ATG6) in the regulation of endothelial cell autophagy during atherosclerosis; it was shown that the elevated expression of the miR-30 can be caused by a high-fat diet that may suppress ECs autophagy protective effects against atherosclerosis development. [42]

II. Conclusion

Present review indicating the compound crucial roles of miRNAs and their relevant molecular mechanisms related to endothelial cells and their dysfunction in atherosclerosis development and progression. The present review concluded that circulating the panel of miRNAs plays as potential role on early identification of endothelial dysfunction associated with CVD. Early prediction was useful to revert of endothelial dysfunction in initial phases of atherosclerosis. These circulating miRNAs can be used as early biomarkers of cardiovascular disease. This is preliminary study which provide the multiple roles of miRNAs on ECs and which set a path for innovative research and for enlighten novel pathological mechanisms and pharmacological targets of atherosclerosis.

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