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miRNA in Pathophysiology of Peripartum Cardiomyopathy (PPCM): A Systemic Review

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Abstract

Peripartum cardiomyopathy (PPCM) was recognized as a clinical entity in the early 1930s; however, the exact mechanism of the disease's progression remains unknown. The highest incidence of PPCM is in African Americans, and the disease is associated with poor outcomes in elderly and multiparous women. The varying characteristics of patients suggest that genetic susceptibility of the disease could exist. PPCM can be associated with life threatening complications including cardiogenic shock, fatal arrhythmias, and thromboembolic events which can lead to death. Pregnant or lactating PPCM patients can further complicate how clinicians manage them. One recent hypothesis is that 16-kDa N-terminal prolactin fragment (16K PRL) plays a vital role in PPCM by inducing microRNA146a (miRNA146a) which reduces angiogenesis through downregulation of NRAS. miRNAs are small RNAs that were previously described as noncoding RNA that controls the posttranscriptional activity of mRNA. Recent animal studies have identified miRNA146a as a causative factor in PPCM; this discovery is promising and could have clinical implications in future. With growing evidence of miRNA's involvement in disease, miRNA can add to our current understanding of pathology and could be a potential tool for diagnosis, prognosis, and therapy in PPCM.

Keywords: MicroRNA/miRNA; miRNA 146a; Peripartum cardiomyopathy; Heart failure; Biomarker

Introduction

The aim of this review is to compile our understanding of microRNA since it was discovered and provide an update on its significance in PPCM. We will discuss various hypotheses that have been presented to explain the pathophysiology of PPCM. PPCM is extremely variable in terms of patient presentation, disease severity, ethnic and gender variation, age of onset, and (most importantly) fluctuating outcomes with similar treatment protocols. While it is known to some extent that genetic susceptibilities exist in PPCM, understanding miRNA's involvement in the disease will add to the present understanding of PPCM. In subsequent sections of the review, we will discuss the clinical implications of miRNA146a in PPCM as a potential biomarker of disease expression as well as its possible therapeutic use.

PPCM

In 1971 Demakis and colleagues introduced the term peripartum cardiomyopathy in reference to 27 women with cardiomyopathy in the puerperal period. The diagnostic criteria included were development of heart failure within the last month or within 5 months of the postpartum period, absence of other identifiable causes of the heart failure, and absence of heart failure prior to the last month of pregnancy [1]. Additional criteria of left ventricular ejection fraction (LVEF) being less than 45% demonstrated by echocardiography and fractional shortening less than 30% were added by the National Heart, Lung, and Blood Institute in 1997 [2]. The definition of PPCM has been recently updated by the European Society of Cardiology: "idiopathic cardiomyopathy presenting with heart failure secondary to left ventricular systolic dysfunction toward the end of pregnancy or in the months following delivery where no other cause of HF is found. The left ventricle (LV) may not be dilated, but the ejection fraction (EF) is nearly always reduced below 45%" [3]. The term pregnancy-associated cardiomyopathy (also known as early pregnancy-associated cardiomyopathy) has been used to describe this condition, and the characteristics of early-onset disease were evaluated in a review of 123 women [4]. 100 women met the traditional criteria for PPCM with a mean of 38 weeks, and 23 women had a mean of 32 weeks. There was no difference between patients who were diagnosed earlier than 38 weeks and patients who were diagnosed 38 weeks or later in terms of clinical presentation and outcome, suggesting that an earlier onset of PPCM is possible [4].

Epidemiology

The incidence of PPCM in the United States varies greatly between different publications from 1:1149 to 1:4075 [5]. In South Africa the incidence of PPCM is 1:1000, and the highest incidence in the world is in Haiti 1:300 [6,7]. Data is collected mostly from single center case series and literature review of case series published online. PPCM incidence is higher in women who have had a history of multiple pregnancies, a history of hypertension, and are older than 30 years. Multiple studies have shown that significantly higher incidences of PPCM exist in African American women [4,6,8]. One of the criteria in

the definition of PPCM is that the disease is non-genetic and non-familial; however, 16% of PPCM patients have a family history of heart failure, suggesting genetic susceptibility could be an important aspect of the disease. Moreover, genetic forms may have lower recovery [9], and it could be used for stratification of patients with PPCM. Prospective, multicenter, and population based epidemiological studies are needed in order to have precise estimation of incidence and early identification of high risk patients.

Pathophysiology in PPCM

During pregnancy there is a significant reduction in systemic vascular resistance in order to accommodate a >40% increase in blood volume. This increase is gradual and occurs during the time between the second and third trimesters of pregnancy [10], resulting in physiological cardiac hypertrophy. It is different from pathological hypertrophy in terms of the histologic feature of fibrosis and reduced LV function later. Balance between cardiac muscle growth and coronary angiogenesis determines physiological *vs* pathological cardiac hypertrophy [11]. In pregnancy the heart develops mild eccentric hypertrophy physiologically [12], and there is a proportionate increase in chamber dimension and wall thickness [13]. In addition to mechanical stress, increase in oestrogen at the end of pregnancy is an additional factor for pregnancy related hypertrophy [12]. The increase in oestrogen, prolactin, and volume overload in combination with increasing stress on the heart could be one of the reasons for development of PPCM as it is commonly seen during last term of pregnancy, but there are no existing studies to support this hypothesis except for prolactin alone as a causative agent of PPCM.

Although it is well understood that physiological hypertrophy of the heart of pregnant women occurs during pregnancy, the process that converts this normal response to pathological hypertrophy and subsequent reduced LV function is unknown. There are several promising theories have been published, but the exact mechanism remains a puzzle. The STAT3 gene is involved in protection of the heart from oxidative stress by up regulating reactive oxygen species (ROS). It is a scavenging enzyme manganese superoxide dismutase [14] that promotes myocardial angiogenesis in a paracrine and autocrine fashion within cardiomyocytes and normocytes [15-17]. Also, STAT3 promotes anti-apoptotic activity via BCL-XL proteins [18]. These findings have been tested in CKO mouse by Hilfiker-Kleiner et al., and they found that cardiac STAT3 levels were markedly decreased in insolated cardiomyocyte fraction but not in normocyte fraction [19]. Increased prolactin levels in pregnant women near term and during the postpartum period are known to increase STAT3 which protects the human heart from oxidative stress [20] by encoding MnSOD and antiapoptotic proteins (BCL-XL). Infusion of prolactin in vivo as well as adding it to the culture of myocytes in vitro activates STAT3. Furthermore, decreased STAT3 protein levels in LVs of PPCM patients have been reported. As previously discussed, these decreased STAT3 levels increase the oxidative stress due to reduced expression of MnSOD leading to increased ROS. Serum oxidized LDL levels were elevated in PPCM patients in comparison to normal pregnant women, indicating that PPCM patients could be experiencing increased oxidative stress [19]. Increased oxidative stress up-regulates the activity of proteolytic Cardiac Cathepsin D which is responsible for cleavage of prolactin hormone (PRL) into 16 kDa. This 16 kDa fragment is anti-angiogenic which reduces capillary angiogenesis and is also responsible for apoptosis causing vascular remodelling and regression of blood vessel. Thus a possible explanation for development of PPCM could be a combined effect of increased oxidative stress and cleavage of prolactin hormone (PRL) into 16 kDa [21-23]. Hilfiker-Kleiner et al. in their experiment found higher 16 kDa PRL levels were detected in 3 out 5 patients with PPCM, and 16kDa PRL levels were not detected in healthy lactating patients [19]. This hypothesis is further tested by experiment using bromocriptine, a D2 receptor agonist known to block PRL release was used in a prospective trial against standard medical therapy (ACE/ARB and BB) results showed higher percentage of patients were labelled as improved in bromocriptine group but results were not statistically significant, and BB and percentage of patients who had complete recovery were similar in both the groups. Some shortcoming were, this is an observational non-randomize trial with smaller power and to test effect of bromocriptine more robust multicenter trial is needed [19]. Another group of researchers Patten et al. conducted experiments to prove cardiac specific PGC-1α knock-out mice develop PPCM [24]. PGC-1α regulates angiogenesis in heart tissue with the help of angiogenic VEGF [25,26]. In late pregnancy, the placenta secretes soluble Flt1 (sFlt1) which reverses the effect of VEGF causing anti-angiogenesis [27]. VEGF-121 injected knockout mice demonstrated improved survival up to 5 pregnancies and a partial increase in capillary density with marginal improvement in LVEF. High SFlt1, multiple gestation, and preeclampsia together create an anti-angiogenic environment particularly during the late period of pregnancy and postpartum where oxidative stress is elevated, therefore contributing to PPCM in addition to the above mentioned STAT3 pathway [24]. However correlation of SFlt1 and PPCM is not established and more prospective trials are needed to define its role in pathogenesis of PPCM.

PPCM is a diagnosis of exclusion, as clinical presentation of left ventricular dysfunction such as ankle swelling, shortness of breath could be considered normal during peripartum period specifically during late pregnancy when PPCM is prevalent(3). In addition due to increased hemodynamic stress during pregnancy, pre-existing cardiac conditions can get worsened, resulting in cardiac dysfunction [3]. Dilated Cardiomyopathy, congenital heart diseases, acquired heart disease during pregnancy; most of these conditions could make diagnosis of PPCM further difficult. Distinguishing characteristic in above mentioned disease state is that, they present during initial terms of pregnancy as opposed to PPCM and one of the diagnostic criteria of PPCM includes absence of identifiable causes of LV dysfunction and normal cardiac function during 1st and 2nd trimester of pregnancy [1,2]. Hypertensive heart disease could manifest as heart failure during pregnancy with distinguishing feature as diastolic heart failure and previous history of hypertension [28]. Bicuspid aortic valve a most common congenital cardiac anomaly with 2% prevalence in general population [29], Atrial septal defects and ventricular septal defects can deteriorate cardiac function during high output state of pregnancy and mimic in presentation with PPCM, however these conditions can be distinguished from PPCM with echocardiography [30].

miRNA biology

miRNAs/microRNAs/ncRNAs are approximately 20 nucleotide long regulators of posttranslational activity of messenger RNA (mRNA). miRNAs play important roles in cell proliferation, differentiation, migration, and apoptosis during normal development and disease progression [31]. One of the ground breaking discoveries of miRNA was made in 1993 by Ambros, Ruykun, and their colleagues by using a nematode *C. elegans* model. A new unexpected cellular regulatory mechanism involving a non-protein coding transcript was found [32,33]. It is predicted that miRNAs regulate approximately

30% of the human protein-coding genome [34]. miRNA was first studied in disease processes in malignancies where miRNA acted as a tumor stimulant or tumor suppressor. Thus, controlling miRNA activity could be therapeutic. miRNAs act by binding to their target mRNAs at 3′-UTRs, using a partial base-pairing mechanism. miRNA at the 5′ end has a sequence of 7-8 nucleotides complimentary to the target mRNA which is called a "seed" region [35]. miRNA acts on its target mRNA by inhibiting the translation or inducing degradation of target mRNA. This is accomplished through the varying number of binding sites, overall degree of complementarity, and accessibility of the mRNA for the miRNA to evoke its effects [36,37]. After activation, miRNAs are released from cells in exosomes to carry out the terminal step in gene expression [38].

Role of miRNA in pathophysiology of PPCM

It has been discovered that 16K PRL activates Nuclear Factor-Kappa B (NF-κB), a signalling pathway in ECs [22] *via* miRNA/microRNA [39]. Halkein et al. further identified the mechanism of how 16K PRL induces PPCM. They found that overexpression of miRNA146a led to an increase in human umbilical vein endothelial cells' (HUVEC) apoptosis, and reduced expression of miRNA146a attenuated apoptosis [39]. The antiangiogenic effect of 16K PRL is carried out by miRNA146a via its novel target, Neuroblastoma RAS viral gene homolog (NRAS) gene, which was identified in the experiment [39]. NRAS was down regulated by miRNA146a in rat heart ECs, reducing angiogenesis. 16K PRL also promotes miRNA146a to be loaded into endothelial exosomes, released from ECs, and then subsequently absorbed by cardiomyocytes. miRNA 146a reduces the metabolism of cardiomyocytes by reducing the expression of its target gene ERBB4 which promotes glucose uptake when stimulated by neuregulin-1 (NRG-1) [40]. In CKO mice with PPCM, an increase in miRNA146a expression was discovered and associated with reduced level of ERRB4. Blocking PRL with bromocriptine in CKO mice with PPCM significantly reduced miRNA146a and increased levels of ERBB4. miRNA 146a in humans with PPCM were found to be elevated, but levels were reduced after a PRL blockade. Further strengthening this experiment, they found miRNA 146a to be normalized when PPCM patients recovered after standard treatment with Beta blockers and ACE inhibitors. Interestingly, a complete blockade of PRL with bromocriptine interfered with lactation while treatment via a blockade of miRNA146a had no effect on lactation. 16K PRL-miRNA146a likely has a crucial role in PPCM, due to the fact that by blocking miRNA 146a in CKO mice, Halkein et al. diminished the occurrence of PPCM to a large extent. They also found enhanced miRNA146a expression in plasma of PPCM patients as compared to healthy pregnant controls, PPCM patients treated with bromocriptine, and patients with dilated cardiomyopathy. While these findings of miRNA146a expression are promising, a single miRNA controls hundreds of genes and each gene is controlled by several miRNAs. Therefore, in order to use miRNA146a as therapeutic agent, more studies are required in order to identify a complete target analysis to anticipate the possible side effects. One possible side effect is an increase in angiogenesis that could be detrimental in several oncogenes [41]. miRNA-15, miRNA-16, miRNA-20a, and miRNA-20b are anti-angiogenic miRNAs that repress VEGF-A like miRNA146a does [42]; however, more experiments are necessary to identify if any of these miRNA could be contributing to anti-angiogenesis in PPCM. Moreover, the STAT3 gene which has been identified to have a vital role in prevention of oxidative stress in cardiac tissue also promotes angiogenesis; conversely, STAT3 seems to be an oncogenic transcription factor in colorectal cancer. It was found that 26 and 21 known miRNAs were significantly overexpressed and under expressed, respectively, in the STAT3-knockdown CRC cell line [43], explaining the wide spectrum of the miRNAs' expression. Results of miRNA146a expression in PPCM are promising, but further studies are needed before we place miRNAs into the current standard of care.

Potential therapeutic approaches of MicroRNA and its limitations

Several ways exist to influence expression of miRNA which include anti-miRNAs and substitution of miRNA by its mimic [44-46]. Each technique has its own benefit and limitation. AntagoMiR or anti-MiRNAs are chemically developed oligonucleotides used for silencing of miRNA activity and are effectively used in mice [44]. These agents could be administered intravenously and has shown significant reduction in corresponding miRNA levels. Recently, inhibition of miR-122 entered in the clinical phase to treat hepatitis C virus infection, multiple other miRNA inhibitors are soon going to be in coming from preclinical phase to clinical phase [47]. However as compared to current modalities of therapeutic agent antiMiRNAs have delayed and prolonged onset of action which limits it's usage in acute disease state. It also means it will accumulate in the tissue for longer period of time and raise concerns for possibility of its toxicity and lack of effective strategies to reverse its effect. Other therapeutic approaches include use of 'miRNA Mimic' in which miRNA target gene is silenced using artificially developed miRNA and causing gene suppression [48]. After administering these miRNA like fragments into the cells they act on specific target and activate RISC complex resulting in gene suppression. Using lipid formulations increased uptake of miRNA mimics is possible and experimentally used in treatment of cancer [49] and is in phase 1 of clinical trial for treatment of hepatocellular carcinoma. It's application in cardiovascular diseases still under preclinical stages. Complexity of miRNA and its target regulation is not yet completely comprehended and further research is required to explore its wide range of effects on multiple organ system. Challenge lays in the fact that lack of specificity in microRNA target and its multiple effects in various organ systems. In other words single miRNA interacts with large number of mRNA, proteins and genes on other hand single protein or gene expression involves numerous miRNAs. And also has smaller inhibitory effect, it becomes further difficult to get complete inhibitory effect using antimiR or its mimic [46,50]. As many of this microRNA are identified in development of malignancy there could be a significant risk in development of cancer [51].

Conclusive Remarks

The field of miRNA is rapidly growing. Its research is no longer limited to oncology, and it has been studied in order to identify its role in various disease processes. There are multiple methods that have been developed to control its expression. A single miRNA controls the expression of more than one gene, and multiple miRNAs are involved in controlling the expression of a particular gene, making the process of understanding the miRNA mechanism more complicated. Accordingly, diminishing or augmenting expression of one miRNA can have unwanted or unanticipated effects. Several ways exist to suppress expression of miRNA which include anti-miRNAs, miRNA inhibitors, miRNA sponges, and its mimic. The path to develop new therapeutics can be quite protracted and difficult. Recently, inhibition of miRNA-122 entered the clinical phase to treat hepatitis C viral

infections, and multiple other miRNA inhibitors are soon going to be transitioning from preclinical phase to clinical phase. miRNAs readily detected in easily accessible extracellular fluids such as blood and urine are currently being explored as biomarkers in a wide range of cardiac conditions. In order to efficiently use miRNAs in clinical practice and replace the current standard of care, rigorous research is needed to understand its molecular mechanisms, the extent of its effects, and its signalling pathways. More than 1800 miRNAs have been identified so far in humans. The miRBase (www.mirbase.org) database is a searchable database of published miRNA sequences and annotations, with more than 1800 miRNAs identified so far in humans [52]. With many new miRNAs being discovered, we will continue to increase our knowledge base about the field of miRNA. miRNA has been identified as a controlling mechanism at the terminal stage of gene expression, and it is quite a promising discovery with a huge impact on various systems, particularly the cardiovascular system and oncology. Understanding miRNA in detail has the potential to close the gap in our lack of understanding of diversity in expression of disease profiles and variable responses to identical management.

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