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MiRNA in Idiopathic Recurrent Pregnancy Loss

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Abstract: Background: Recurrent Pregnancy loss is defined as the spontaneous end of a pregnancy before the age of fetal viability and encompasses all losses of conception until 20–24 weeks gestation. Guidelines from the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) define recurrent pregnancy loss as the failure of two or more clinically recognized pregnancies. MiRNAs are small non-coding RNAs, with an average 22 nucleotides in length. Most miRNAs are transcribed from DNA sequences into primary miRNAs (pri-miRNAs) and processed into precursor miRNAs (pre-miRNAs) and mature miRNAs. In most cases, miRNAs interact with the 3'UTR of target mRNAs to suppress gene expression. As well as their established and emerging roles in normal pregnancy, miRNAs through their ability to dynamically regulate gene expression are also believed to contribute to pregnancy-related pathologies including implantation failure and recurrent pregnancy loss, pre-eclampsia, preterm labor, as well as intrauterine growth restriction. It is well-recognized that consistent failure of endometrial receptivity for embryo implantation may have an immunological basis with altered immune cell and cytokine populations being observed in the endometrium of females with a history of recurrent pregnancy loss

Keywords: *MiRNA, Idiopathic Recurrent Pregnancy Loss*

Introduction

Recurrent Pregnancy loss (RPL) is defined as the spontaneous end of a pregnancy before the age of fetal viability and encompasses all losses of conception until 20–24 weeks gestation [1, 2]. Guidelines from the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) define RPL as the failure of two or more clinically recognized pregnancies [3, 4]. Of note, ectopic pregnancies and molar pregnancies are not included in the definition of RPL.

Unlike sporadic pregnancy loss, RPL requires medical intervention encompassing access to specialists, investigations, and enhanced support and follow-up during future pregnancies [4].

Incidence of Pregnancy Loss

The incidence of early pregnancy loss depends on the method used to detect pregnancy. About 50% of all pregnancies are lost at preclinical stages due to biochemical loss or implantation failure [5, 6]. The incidence in clinically diagnosed pregnancies is about 9–20% [6, 7], occurring mainly during the first trimester (weeks

5–12 of gestation) [8]. Week-by-week miscarriage rates vary in early pregnancy, with studies reporting a sharp reduction after 12 weeks of gestation to an incidence of about 1% [8].

Prevalence of Pregnancy Loss

No geographical variation in the prevalence was found in one meta-analysis [9]; however, cultural and societal attitudes may cause women to not openly confess their pregnancy loss, leading to the underestimation of prevalence [10, 6].

MicroRNAs (miRNAs)

miRNAs are small non-coding RNAs, with an average 22 nucleotides in length. Most miRNAs are transcribed from DNA sequences into primary miRNAs (pri-miRNAs) and processed into precursor miRNAs (pre-miRNAs) and mature miRNAs. In most cases, miRNAs interact with the 3′UTR of target mRNAs to suppress gene expression [11]. Interaction of miRNAs with other regions, such as the 5′UTR, coding sequence, and gene promoters, have also been reported [12]. Moreover, miRNAs have been shown to activate gene expression under certain conditions [13]. miRNAs are important for normal animal development and are involved in different biological processes [14]. Aberrant miRNA expression is associated with many human diseases [15]. Furthermore, miRNAs are secreted into extracellular fluids, and these extracellular miRNAs have been reported as potential biomarkers for a variety of diseases, and they also serve as signaling molecules to mediate cell-cell communications [16].

Biogenesis of miRNAs

The biogenesis of miRNA is classified into canonical and non-canonical pathways (Figure 1).

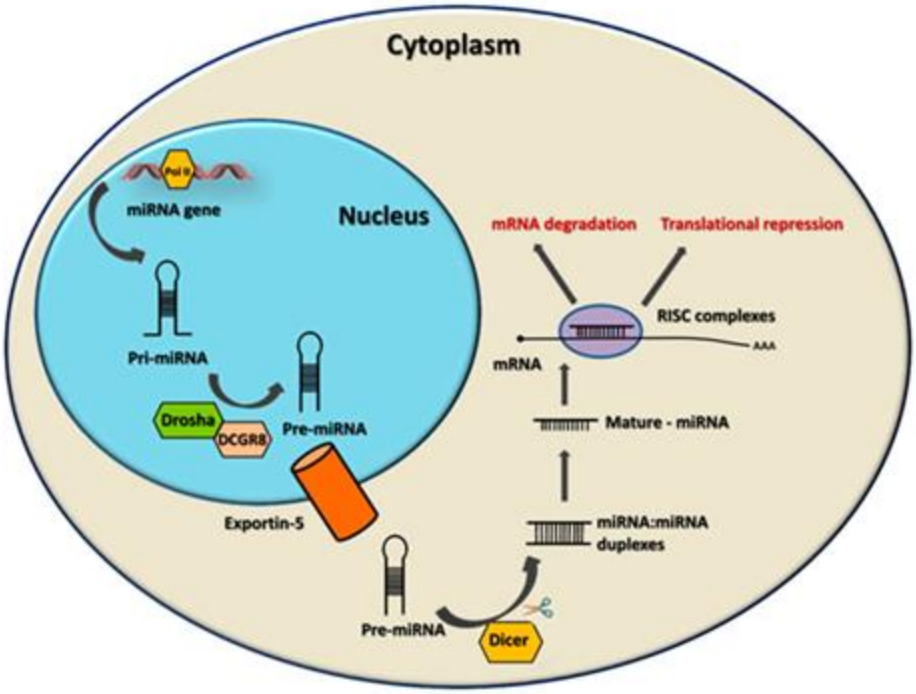

Figure 1: Biogenesis of microRNA [17].

The Canonical Pathway of miRNA biogenesis

The canonical biogenesis pathway is the dominant pathway for miRNA processing. In this pathway, pri-miRNAs are transcribed from their genes and then processed into pre-miRNAs by the microprocessor complex, consisting of an RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8) and a ribonuclease III enzyme, Drosha [17]. Once pre-miRNAs are generated, they are exported to the cytoplasm by an exportin 5 (XPO5)/RanGTP complex and after that processed by the RNase III endonuclease Dicer [18]. This process results in a mature miRNA duplex [19]. The directionality of the miRNA strand determines the name of the

mature miRNA form. The 5p strand arises from the 5′ end of the pre-miRNA hairpin while the 3p strand originates from the 3′ end. Both strands derived from the mature miRNA duplex can be loaded into the Argonaute (AGO) family of proteins (AGO1-4 in humans) in an ATP-dependent manner. The selection of the 5p or 3p strand is based in part on the thermodynamic stability at the 5′ ends of the miRNA duplex or a 5′U at nucleotide position 1 [20].

Non-canonical miRNA biogenesis Pathways

Multiple non-canonical miRNA biogenesis pathways have been reported. These pathways make use of different combinations of the proteins involved in the canonical pathway, mainly Drosha, Dicer, exportin 5, and AGO2. In general, the non-canonical miRNA biogenesis can be divided into Drosha/DGCR8-independent and Dicer-independent pathways. Pre-miRNAs produced by the Drosha/DGCR8- independent pathway resemble Dicer substrates. These RNAs are directly exported to the cytoplasm through exportin 1 without the need for Drosha cleavage. On the other hand, Dicer-independent miRNAs are processed by Drosha from endogenous short hairpin RNA (shRNA) transcripts [21]. These pre-miRNAs require AGO2 to complete their maturation within the cytoplasm because they are of insufficient length to be Dicer-substrates. This in turn promotes loading of the entire pre-miRNA into AGO2 and AGO2-dependent slicing of the 3p strand. The 3′-5′ trimming of the 5p strand completes their maturation [22].

MicroRNAs as Biomarkers

The diagnostic potential of miRNAs has become one of the most important applications in medicine. Previously, investigators believed that RNA molecules could not be employed as biomarkers by measurement in blood samples, due to the relatively high levels of nucleases in plasma that potentially degrade nucleic acids. However, with the discovery of miRNAs that are stable in samples of fixed tissue, the idea became applicable. Moreover, miRNAs can be easily extracted from human body fluids, making them readily accessible [23].

miRNAs have shown satisfactory specificity for specific tissues or cell types (for example, miRNA-122 is expressed only in the liver, miRNA-124 is brain-specific, and miRNA-208 is exclusive to the heart) and good sensitivity for disease progression state [24].

Disruption in miRNA regulation can affect a wide range of diseases, including malignant diseases, inflammatory diseases such as rheumatoid arthritis (RA), cardiovascular disorders, and developmental disorders, which makes these small molecules interesting biomarkers [25].

Among immune system-related miRNAs, miRNA-21, miRNA-146a, and miRNA-155 are the most intensively studied miRNAs that have been demonstrated to regulate immune responses and tissue inflammation in allergic diseases. These circulating miRNAs could be ideal blood biomarkers due to their disease-specific dysregulation and their relative stability compared with mRNAs [26].

Role of miRNAs and Immune Regulation in Conception

There is extensive evidence supporting the contribution of specific miRNAs to the functional capacity of immune cells [27]. Indeed, miRNAs play a critical role in immune function with alterations to both innate and adaptive immune responses, with systemic compromise in T cells observed following depletion of miRNAs by mutation [28].

miRNAs and Regulatory T cells (Treg cells)

Treg cells have a distinct miRNA profile compared to naïve CD4+ T cells, suggesting that miRNAs contribute to Treg cell differentiation and function [29]. Evidence for miRNA regulation of the endometrial immune environment at conception can be seen in individuals who go on to develop immune-associated pathologies of pregnancy, where altered first trimester and pre-conception immune-regulatory miRNAs are indicative of later pregnancy outcome [30]. Various genes and molecules contributing to the tolerogenic immune environment are differentially controlled by miRNAs, such as human leukocyte antigen (HLA)G, which is regulated by miR-152, miR 148a, and miR133 [31].

miRNAs and Phenotype and Function of Antigen-Presenting Cells (APCs)

APCs, including macrophages and dendritic cells (DCs), are master regulators of tolerance, through promoting the expansion of Treg cells [32]. APCs are abundant at ovulation with exposure to seminal fluid enhancing their

recruitment [33] and differentiation into a tolerogenic phenotype within the female reproductive tract and local lymph nodes [34].

In DC development, miRNAs coordinate differentiation from monocytes, with 20 miRNAs, including miRNAs previously implicated in myeloid cell differentiation (miR-20a, miR-17-5p, and miR-106a) exhibiting stage-specific differential expression. Inhibition of those DC-enriched miRNAs alters DC differentiation with miR-34a having the most prominent effect [35].

In addition, endometrial DCs in the periconception period have the capacity to differentiate into a tolerogenic DC (tDC) phenotype. In pregnancy, tDCs are implicated in promoting Treg cell differentiation and suppressive function [36]. miRNAs contribute to this process as studies have shown stage-specific miRNA profiles distinguishing immature, activated, and tDCs in humans. Particularly, miR-17, miR-133b, miR-203, and miR-23b are uniquely elevated in human tDCs and are predicted to play an important role in tDC differentiation [37].

miRNAs are also considered important in the activation of phenotypes of macrophages [38]. Macrophages can exhibit both pro-inflammatory (M1-like macrophages) and anti-inflammatory (M2-like macrophages) properties. miR-155 expression is increased in M1 compared to M2 macrophages [39] and promotes M1-like polarization and function through the downregulation of the anti-inflammatory suppressor of cytokine signaling 1 (Socs1) [40]. In contrast, miRNAs such as miR-223 and let-7c may promote M2 macrophage polarization [41].

miRNA in Idiopathic Recurrent Pregnancy Loss

As well as their established and emerging roles in normal pregnancy, miRNAs through their ability to dynamically regulate gene expression are also believed to contribute to pregnancy-related pathologies including implantation failure and RPL, pre-eclampsia, preterm labor, as well as intrauterine growth restriction [42]. It is well-recognized that consistent failure of endometrial receptivity for embryo implantation may have an immunological basis with altered immune cell and cytokine populations being observed in the endometrium of females with a history of RPL [43].

Testing miRNA levels in villous and decidual tissue revealed that many miRNAs were differently expressed in RPL compared to the control group. miR520f, miR3175, and miR4672 were downregulated, while miR517c, miR591a-1, miR522, miR520h, and miR184 were found upregulated in RPL women [44]. Isolated natural killer (NK) cells from decidua tissue of both women suffering from idiopathic RPL (IRPL) and healthy controls identified six differentially expressed miRNAs: miR-34a, miR-155, miR-141, miR-125a, and miR-125b were upregulated, while miR-24 was downregulated, in the IRPL group, compared with those in healthy controls [45].

Variable expression of circulating miRNAs was also detected in plasma of women suffering from IRPL and healthy controls, as miR320b, miR146b-5p, miR221-3p, and miR559 were upregulated, whereas miR101-3p was downregulated [46].

miR-133a is involved in the pathogenesis of RPL by affecting the translation of HLA-G [47]. HLA-G is a nonclassical major histocompatibility complex which is expressed in the placenta during the full length of gestation and almost solely in the extravillous trophoblasts at the fetal-maternal interface [48]. The peculiar localization of HLA-G suggests that it plays a crucial role in the maternal immune tolerance to the fetus. Decreased HLA-G expression was detected in women with RPL when compared with the control group [47].

miR-155-5p and miR-146a-5p were detected to be involved in the regulation of NF-κB1. NF κB1 regulates innate and adaptive immunity and induces inflammation by stimulating the expression of pro-inflammatory genes, especially IL-6 and IL-8 [49]. In addition, miR-146a negatively regulates the expression of IL-6 and IL-8 [50].

A strong link between miR-146a-5p and hsa-miR 21-5p, and with NF-κB1 detected, predicting that NF κB1 interaction with hsa-miR-146a-5p affects IL-8 expression, suggesting feedback loops involving NF-κB1 and hsa-miR-146a-5p or hsa-miR-21-5p for controlling RPL-associated inflammation [50].

miR-21-5p was shown to be activated by IL-6 in a STAT3-dependent manner [51]. STAT3 is a protein involved in cytokine and growth factor signaling. It is a key regulator of the anti-inflammatory signaling pathway. Altered STAT activity was implicated in adverse pregnancy outcomes [7].

Methods for miRNA Detection

Methods for miRNA detection include:

Quantitative Polymerase Chain Reaction (qPCR): Most widely used method for the detection of miRNA [52].

Northern Blotting: A hybridization-based technique where isolated miRNA is separated by gel electrophoresis, transferred to a membrane, and detected by hybridization with a DNA or RNA probe [52].

Microarray Analysis: Microarray technology is a developing technology used to study the expression of many genes at once. It involves placing thousands of gene sequences in known locations on a glass slide called a gene chip. A sample containing DNA or RNA is placed in contact with the gene chip. Complementary base pairing between the sample and the gene sequences on the chip produces light that is measured. Areas on the chip producing light identify genes that are expressed in the sample [52].

MicroRNA Inhibition and Replacement Therapy

The central action of miRNAs is to restrain gene expression by binding and silencing specific mRNAs, which in turn modulates cellular function. Accordingly, miRNA-based gene therapies in RA aim to artificially interfere with target miRNA activity, managing RA-related gene expression and protein synthesis via the silencing of overexpressed pathogenic miRNAs and increasing the expression of underexpressed beneficial miRNAs [53].

Clinical studies of miRNA-based therapeutic strategies mainly use inhibitors and mimics of miRNAs. miRNA inhibitors are synthetic single-stranded RNA molecules consisting of nucleotides complementary to endogenous miRNAs, whereas miRNA mimics are synthetic double-stranded RNAs that bypass the synthesis process of endogenous mature double-stranded miRNAs and achieve high expression of specific miRNAs in a short duration [54].

Conclusions: The dysregulation of miRNA expression is strongly associated with recurrent miscarriages. The circulating in the peripheral blood miRNAs, miR-100-5p and let-7c, might be utilized as biomarkers and establish a valuable non-invasive prognostic and diagnostic tool in the future.

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