Clinical and Biological Importance of Micro RNA in the Formation of Women Reproductive Losses

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Abstract

Habitual miscarriage is currently one of the most frequent complications of the gestational process, which is diagnosed in 10-25% of pregnant women. (IuA, 2017) Habitual abortion (miscarriage) - spontaneous interruption of pregnancy two or more times in a row.

So, high expression of miR133a may mediate a decrease in HLA-G expression at the protein level and may be implicated in the pathogenesis of habitual miscarriage, since HLA-G provides immune tolerance in pregnancy[49]. So, the literature data suggest that microRNA dysregulation may be related to the pathogenesis of various obstetric complications, but accurate methodological approaches to the diagnosis and prediction of pregnancy complications using microRNA require further study.

Keywords: *Microrna, pregnancy, placenta, gene.*

Introduction

In some countries, three or more abortions are considered habitual, but examination for the cause of the abortion is recommended after two abortions[2]. The frequency of Habitual miscarriage in the population is up to 5% of pregnancies. In the structure of miscarriage, the frequency of miscarriage is up to 20%. Habitual miscarriage is a polyethological complication of pregnancy based on reproductive disorders[1] The causes of miscarriage, both episodic (single or repeated) and recurrent (loss of three or more consecutive pregnancies), are similar. They can be combined into the following groups: genetic, infectious, endocrine, anatomical, immunological, and idiopathic[3] Biomarkers, which are present in the mother's serum, will be the most valuable, as obtaining the mother's blood is a safe and affordable method, and blood can be obtained in sufficient quantities.[4] The exchange of information between the functional systems of the mother and fetus is important for normal pregnancy, fetal development and growth. This connection reduces the risk of rhesus conflict and coordinates biological resources for the benefit of both organisms. Previously, the mother-fetal link was thought to provide hormones and growth factors that are soluble in the blood or bound protein carriers and serve as paracrine or endocrine signals to control healthy pregnancy. It is now clear that nucleic acids, and especially small RNA molecules, circulate between tissues and affect them at the cellular level.

The main class of non-coding RNA and one of the most well studied is the family of small regulatory RNA called micro-RNA. The term micro-RNA was first introduced in the early 2000s. Micro-RNA were originally described in the Caenorhabditis Elegans nematode in 1993 and later were found in the genome of many organisms, including humans. The human genome encodes more than 1000 micro-RNA species and about 60% of the human and other mammalian genes are targeted[5]

Micro-RNA's (miRNA's) are endogenous RNA's ranging in size from 20-22 nucliotides that can play

an important regulatory role in animals and plants by targeting mRNA's for cleavage or translational repression. Oligonucleotide micromatrixes have shown that microRNAs are highly enriched in the placenta. (Wu et al., 2012) RNA-induced jamming complex to the RNA target, with subsequent weakening of gene expression by inhibiting matrix RNA (mRNA) translation and/or RNA degradation. This is accomplished by complementary binding their «seed sequence» to the target in the 3'-nontransmitted mRNA region either by detaching the Cap 5'-structure (decapitation) or deadenylating the poly(A)tail. Consequently, they participate in transcription and posttranscriptional regulation of gene expression and play an important role in various biological processes[6].

Cells transcribe microRNA precursors (primary transcripts) (PRI-microRNA), which undergoes multistage biogenesis with the formation of mature microRNA 18-25 nucleotides in length. The synthesis of microRNA begins in the nucleus, with RNA polymerase II-mediated transcription, using genomic DNA as a matrix. A long primary microRNA (known as PRI-microRNA) is then formed, which forms a complex secondary structure by folding loops and studs.) The dual-chain structure of RNA can be easily recognized by the nuclear protein DGCR8, which functions in combination with Drosha, the protein that cuts through RNA, due to the catalytic domain of RNAase III, forming a precursor microRNA molecule in the form of a stud. PRE-microRNA can bind to the nuclear transport factor, the exporter-5, and is exported to the cytoplasm by the energy of guanosine triphosphate. In cytoplasm, PRE-microRNA is broken down by endonucleases specific to double-stranded RNA, resulting in the formation of microRNA: duplex of ~22 nucleotides in length. As a rule, only a few can bind to Argonaute proteins in the RNA-induced Gene Jamming Complex (RISC) and participate in the RNA interference process. The remainder is destroyed by the gene jamming complex. miRNA's specifically expressed in human placenta were found in the sera of pregnant women and were found to be significantly elevated in comparison with those of non-pregnant women; their levels increased with gestational age and decreased after delivery, providing a new group of molecular markers for monitoring pregnancy[7].It should be noted that the expression profile of tissue-specific and disease-associated microRNA's is more selective and stable than the general mRNA profile. The biological efficacy of specific mRNA's is in their subsequent conversion into specific proteins as compared to microRNA's, which act as key regulators of polymer genes. So, microRNA more accurately reflects physiological changes in the body. After long researches it was proved that microRNA is everywhere present in body fluids and the mechanism of genetic exchange between cells in horizontal order takes place. Circulating or extracellular microRNA's are more stable and protected against RNAase degradation. This protection is achieved either by complexes with different proteins or by inclusion in different types of extracellular vesicles. MicroRNA's have been shown to be ten times more stable than mRNA's, which have a half-life expectancy of ~10 hours. The average half-life of microRNA, according to a mathematical model, is 119 h and some microRNA's, such as 125b microRNA (half-life, 225 h), are more stable than others. This is probably due to their inherent structural features[8]. The use of single-primer real-time PCR or real-time PCR using high-capacity platforms ensures that quantitative analysis of microRNA from tissue or plasma/serum will be easier and more accurate. This highlights the ease of using microRNA as a biomarker for the development of practical method to detect obstetric pathology. MicroRNA analysis shows that tissue diversity is reflected in the microRNA expression profile. In addition, the expression profile of pathological tissue differs significantly from that of normal tissue, which can be useful for clinical diagnostics. So, aberrant microRNA expression is associated with cellular dysfunction and a number of pathological conditions such as cancer, inflammatory and immune diseases, cardiovascular disorders[9]. Abnormal expression of miRNA's has been observed in multiple diseases of the human reproductive tract, including preeclampsia, endometrial endometrial adenocarcinoma, leiomyoma, and ovarian cancer, endometriosis, and pregnancy recurrence. The following review provides an update on the role of microRNA and gynaecological diseases, covering not only the impact of microRNA regulatory violations on the origin of each disease, but also shows the potential useful diagnostic and therapeutic tool that miRNA can play in these gynecological pathologies[10]

The increased expression of specific microRNA's causes a repressive transmission from the mRNA target, while the decreased expression of these microRNAs has the opposite effect. These changes in mRNA translation define a distinct protein expression profile, affecting multiple molecular pathways, and induce many different

cell and tissue changes. Thus, the cumulative effect of multiple microRNAs can affect human health as well as determine the outcome of a particular disease. These data may allow for a better understanding of changes associated with genital tract diseases and may be useful for developing newer therapeutic approaches as well as less invasive and more effective diagnostic tools[11]. MicroRNAs play an important role in regulating many events, such as inflammatory and immune responses, cellular cycle, differentiation, apoptosis and tissue remodeling. The precise regulation of the expression of these genes is fundamental to the normal functioning of the endometrium. Thus, dysregulation of microRNA expression is associated with certain gynecological pathological processes such as endometriosis, leiomyoma and habitual miscarriage[12]. The microRNA expression profile has been shown to be more important than the mRNA expression profile for discriminating normal and pathological tissues. As a result, microRNA may have a significant impact on the development of new and more accurate diagnostic method as well as more effective therapeutic approaches. A number of microRNAs specific to the human placenta have been detected using the Real Time Polymerase Chain Reaction (PCR-RT) method, each of which has an individual expression level[12]. Some specific microRNA (microRNA516-5p, 517*, 518b, 520a*, 520h, 525 and 526a) are considered pregnancy-specific microRNAs. The importance of mRNA in early embryo development has been identified in a number of species, from Caenorhabditis elegans to mammals.[13-18]It has been shown that in these organisms miRNA-supplied gene regulation is necessary for normal embryogenesis, as evidenced by the reduced survival of embryos in mutant embryos.

The importance of microRNA is confirmed by the fact that deletion of Dicer1 gene (Dcr-1) in mice leads to stopping embryo development on 7.5 days due to differentiation defects[19]. Jan et al.[20] reported similar effects between 12.5 and 14.5 days of pregnancy due to altered embryonic angiogenesis, which also appears to be regulated by microRNA. These researchers have demonstrated that, since miRNAs act as posttranscriptional suppressors of target genes, a defect in Dicer leads to defects in miRNAs. This, in turn, causes the over-expression of target genes such as Ang-1 and PTEN in embryos. In the same way, Medeiros et al [21]. have demonstrated that miRNA Mir-290-295 deficiency leads to partial embryo death and germ cell defects. Micro-RNAs are also highly expressed in human embryonic stem cells. [23]. Rehn and Co. found that miR-520 may be closely involved in human embryonic cell function. Other miRNAs, such as miR-302 and miR-200 families, were similarly observed in human [30] and mouse embryonic stem cells[31]. Rosenbluth et al.[34] studied 754 microRNAs, were expressed in 14 blastocysts (five females, four males and five aneuploid embryos) with matrix screening. They confirmed using real-time quantitative PCR (RTR) the most expressed microRNAs including miR-372, miR-720 and miR-302c in 27 blastocysts (seven males, eleven females and nine aneuploids). These researchers observed that human blastocysts express a large number of microRNAs, which are necessary for the survival and development of embryos, as well as to preserve pluripotency of stem cells. Most of them have already been registered in human embryonic stem cells[29] mammalian embryos[35] or primate placenta[37]. Moreover, when comparing male and female embryos, these researchers found that one miRNA, miR-518d-5p, was expressed 5.6 times in male embryos, indicating some degree of sexual differentiation even at the blastocyst stage. Interestingly, Mir-518d-5p mRNA belongs to C19MC cluster, the largest human cluster where miRNA genes are organized. In these clusters, miRNA genes are expressed only in the placenta and can participate in the development and implantation of the placenta. Similarly, miRNA expression was found to be higher in mouse embryos than in mature tissues, confirming their role in embryo development [38]. More recently, Rosenbluth et al.[39] have demonstrated that miRNA can be detected in In Vitro Fertilization (IVF) culture media where they are differentially expressed according to the fertilization method, chromosome status and pregnancy outcome. These results show that miRNAs are potential markers for predicting success after IVF[40].

Concentrations of some other microRNAs are significantly higher in plasma in pregnant women than in non-pregnant women. Moreover, the concentration of some microRNAs (e.g. microRNA 141) increases with increasing gestation period[41]. The highest intensity of expression in the placenta is typical for microRNA 517a, 517b, 516b, 525 -5p, 512-3p, 515-3p[42]. It is also noted that microRNA 517a-3r, 519 a-3r, 520 s-3r are determined in mesenchymal stromal cells of the placenta, being an indicator that the specific functions of the placenta are not limited only to the trophblast. At the same time, the intensity of microRNA expression of 125b5p in the trofooblast is much higher in the third trimester compared to I. The different expression of microRNA patterns in the nap tree of the placenta in

the first and third trimesters of pregnancy highlights the individual profiling of microRNA and ensures normal development of the placenta. Therefore, the level of microRNA expression can be an indicator of placenta changes during pregnancy depending on the period of gestation and development of the choroid branches[8]. The search for microRNAs that could serve as biomarkers of complications during pregnancy was initiated by Chim and colleagues in 2008. At the time, about 100 microRNAs were known to be expressed primarily in the placenta, but it was not known whether microRNAs obtained from the placenta would be detected in the mother's blood and useful in diagnosis. In a landmark study, Chim and his colleagues suggested that most plasma microRNAs, which are not pregnancy-related, originate in the hematopoietic compartments, and therefore they compared 157 microRNA profiles isolated from the placental tissue in the third trimester of pregnancy with microRNA isolated from the mother's blood cells. They identified 34 microRNAs that were present in the placenta at concentrations tens of times greater than those in the maternal blood cells. Importantly, the researchers additionally showed that the four most common of these placental microRNAs (microRNA-141, microRNA-149, microRNA-299-5p, and microRNA-135b) were found in the mother's plasma during pregnancy but were not found in postpartum plasma, another confirmation that they were of placental origin. Placental specific microRNAs were subsequently detected in plasma and serum and in a number of other studies. These data clearly show that placental microRNAs are present in the maternal bloodstream[8]. It was originally thought that nucleic acids (DNA and RNA) of placental origin enter the maternal bloodstream as apoptosis cells. Lo et al. have shown that microRNAs are exported from the human placental syncytial trophoblast to the maternal bloodstream through exosomes, and suggested that circulating microRNAs derived from the trophoblast reflect the physiological state of pregnancy and can be used for diagnostic purposes. Because their concentrations probably reflect tissue-specific physiological or pathological conditions, many recent studies have focused on identifying microRNAs that correlate with them and thus allow predicting complications of pregnancy. As for the normal human placenta, the expression of microRNA belonging to the C19MC, C14MC and miR-371-3 clusters depends on the gestational period. C14MC, the largest microRNA cluster containing 52 microRNA genes, is preserved without significant structural changes and is unequivocally determined in placental mammals. This suggests the important role of C14MC microRNA in controlling neurogenesis, embryonic development, transcription regulation and RNA metabolism. The C19MC cluster of 46 microRNA genes plays an important role in human embryonic development in the same way as the insulin-like growth factor-2 (IGF-2). It should be noted that some of the C19MC cluster microRNAs were found in the maternal bloodstream during pregnancy, including microRNA-515-3p, microRNA-516-5p, microRNA-517A, microRNA-517c, microRNA-518B, microRNA-520a *, microRNA-520h, microRNA-526a and microRNA-526b. Thus, maternal, circulating microRNAs may have the potential to become a new diagnostic tool for pregnancy pathology[47]. The cluster of microRNA-371-3 consists mainly of three microRNAs, HSA-Mir-371- 5p, HSA-microRNA-372 and HSA-Mir-373-5p and is located on chromosome 19. The cluster mainly expresses itself in the placenta, and the amount of its microRNA decreases in the process of development, therefore it seems to be important for the maintenance and regulation of proliferation and apoptosis. In addition, it was found that microRNA's from the cluster microRNA-17-92 are expressed in the normal motensive placenta. These microRNA's are important for angiogenesis and affect the expression of many genes, namely HIF1A, interleukin-8, tissue inhibitor metalloproteinase-2, metallopeptidase-2 matrix, VEGFA, efrin-B, and efrin receptor B4. The abundant expression of microRNA in amniotic fluid has been confirmed; it contains mainly those microRNA's that are involved in supporting embryonic development and immunity, thus protecting the fetus from external pathogens and the mother's immune response[44]. Some microRNAs with oncogenic and immunologic suppressor characteristics, such as cluster microRNA-17-92, microRNA-371, C14MC, and C19MC, mentioned above, have been found to express themselves more strongly in the first trimester of pregnancy, a microRNA of oncosupressors and those microRNAs that promote differentiation and have hematopoietic activity, including LET-7 family microRNA and cluster microRNA-29 and microRNA-34, are more strongly expressed in the third trimester of pregnancy. In addition, aberrant expression of some microRNA's has been recorded in placenta or lint in women with pathological pregnancy. Expression of HSA-MIR-184, HSA-MIR-187 and HSA-MIR-125b-2 in the lint of patients with normal pregnancy loss has been found to be significantly increased, while expression of HSA-MIR-520F, HSA-MIR-3175 and

HSA-Mir-4672 has been significantly reduced compared to that of women with normal pregnancy.

Studies in the laboratory have shown that microRNA is involved in the regulation of proliferation, migration, invasion, apoptosis and angiogenesis of trophoblast cells. Thus, it is quite probable that microRNA is necessary for normal development of the placenta and that pathological expression of microRNA may lead to defective placenta and abnormal pregnancy, resulting in miscarriage[46]. It has been shown that seven specific placental microRNAs present in maternal plasma (microRNA-516-5p, microRNA-517, microRNA-518b, microRNA-520a, microRNA-520H, microRNA-525 and microRNA-526a) are associated with pregnancy and may have diagnostic potential. There is a direct correlation between the level in maternal plasma of extracellular pregnancy-associated placenta-specific microRNA-515-3p, MIR-517A, MIR-517c and microRNA-518B and placental mass. The relationship between placental preposition and extracellular pregnancy-associated placental-specific microRNAs in the mother's plasma has been identified. They found significantly higher concentrations in plasma of extracellular MIR-517A and significantly lower concentrations in plasma of extracellular MIR-518B in the placenta predestination group in comparison with the control group and suggested that the level of circulating extracellular MIR-517A can be a prognostic marker of the risk of bleeding at late stages of pregnancy and massive bleeding at delivery. Other studies have provided evidence that functional oligonucleotide polymorphisms (OP) of microRNA-125 may increase susceptibility to habitual miscarriage. Two variants of the allele in primal-microRNA-125 have been identified, namely rs41275794 and rs12976445, which lead to changes in microRNA-125 production. Genes of inhibitory factor leukemia receptor (LIFR) and ERBB2 are targets of microRNA-125. LIF is a gene involved in the growth of the trophoblast and differentiation of the human placenta, which, like ERBB2, regulates the proliferation of stroma cells and differentiation of the trophoblast cells in the placenta in the period after implantation. In addition, ERBB2 can stimulate angiogenesis and invasion by increasing the concentration of vascular endothelial growth factors (VEGF). All these data suggest that these genes may play an important role at early gestational ages and their disruption may lead to PPB in some patients.

Expression of microRNA-125 A-T haplotype decreases in microRNA-125, which leads to increased expression of LIFR and ERBB2. Apparently, ectopic expression of LIFR may partially block LIF activity and cause disturbances of trophoblast growth and differentiation during the post implantation period, which leads to the usual loss of pregnancy. Finally, further studies have investigated associations of microRNA polymorphisms including MIR-146aC> G, MIR-149T> C, MIR-196a2T> C and MIR-499A> G in women with habitual miscarriage. Patients suffering from miscarriage showed a higher frequency of microRNA-196a2CC and microRNA-499AGþG genotypes compared to the control group of healthy patients. The combination of both mutations showed synergistic effects; all these polymorphisms are largely related to idiopathic loss of pregnancy.

Functional genomics has been widely studied, and recently endometrial gene expression signature has been used for implant window screening. Endometrial microRNA has been identified in recent years, during the middle secretory phase, and it is assumed that it regulates cell proliferation and differentiation, which may affect embryo implantation, but their exact role is not yet understood.

In order to study microRNA (miRNA), a prospective cohort study including 12 good female infertility predictions and endometrial gene expression tests during natural cycles before IVF treatment, ERA analysis revealed 5 receptive and 7 post-receptive endometrial samples. No depressed miRNA's were found comparing receptive and post-receptive endometrium. However, eight miRNA's were found to be activated with a multiple of more than 3.0 changes in susceptible samples. Among them, two positively regulated miRNA's were statistically significant: has-miR-374a-5p (multiple: 8.9, $p = 0.03$) and has-miR-17-5p (multiple: 8.9, $p = 0.03$). Despite their insignificance, the other two miRNAs had high multiples of has-miR-30a-5p (multiples of 9.7, $p = 0.116$) and has-miR-103a-3p (multiples of 10.5, $p = 0.125$). Eight microRNAs have been identified as being differently compressed in the receptive endometria by ERA analysis and may be considered as new potential candidate biomarkers for endometrial susceptibility.

Researches show that miR-374a and miR-103 family members are miRNA's associated with cell proliferation, mainly in cancer, while miR-17 and miR-30 family members were identified in the uterine cavity during the implantation window and may be associated with endometrial susceptibility[48].

Recent studies have evaluated the effect of microRNA on the regulation of the HLA-G gene in habitual miscarriage. HLA-G is a non-classical antigen of the main Histocompatibility Complex (MNS) and is expressed almost exclusively on the surface of trophoblast lint in the transplacental barrier during pregnancy, playing a crucial role in the immune tolerance of the mother to the fetus. After microRNA microchipping in habitual miscarriage patients and a control group of patients undergoing planned abortions, it was found that excess expression of microRNA-133a was found in abortions with abnormal karyotype. Further analysis showed that HLA-G is the target of miR133a. It is regulated by binding to a 3'' untranslatable area of HLA-G.

So, high expression of miR133a may mediate a decrease in HLA-G expression at the protein level and may be implicated in the pathogenesis of habitual miscarriage, since HLA-G provides immune tolerance in pregnancy[49]. So, the literature data suggest that microRNA dysregulation may be related to the pathogenesis of various obstetric complications, but accurate methodological approaches to the diagnosis and prediction of pregnancy complications using microRNA require further study.

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