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MicroRNA Biomarkers for Early Detection of Embryonic Malformations in Pregnancy

Xuezheng Li[1] and Zhiyong Zhao[2]

[1]*Department of Pharmacy, Affiliated Hospital of Yanbian University, Yanji, Jilin, China*
[2]*Department of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, Maryland, USA*

Abstract

Congenital birth defects, manifested in newborn infants, are formed during early embryogenesis. Targeted and individualized interventions to prevent birth defects require early detection of risk and signs of developmental abnormalities. Current diagnosis of structural anomalies largely relies on ultrasonography, which can only detect abnormities after their formation in fetuses. Biomolecules, mainly proteins, in maternal blood have been used as indicators of fetal anomalies; however, they lack adequate sensitivity for detecting embryonic malformations. Recently, cell-free microRNAs (miRNAs) have been found in blood and evaluated as biomarkers for diseases. Expression of certain miRNAs in maternal plasma has been shown to be correlated with birth defects in infants. Although their reliability and sensitivity remain to be validated, miRNAs, which can be amplified and sequenced, are potentially sensitive and specific biomarkers for early embryonic dysmorphogenesis.

Keywords: miRNA; Birth defect; Embryonic malformation; Diagnosis

Introduction

Birth defects are a serious public health problem. Every year, nearly 270,000 newborns die and 3.3 million children suffer from congenital anomalies worldwide [1]. In the United States of America, where perinatal care is widely available, about 120,000 babies are born with structural abnormalities [2]. The causes of developmental malformations include genetic and non-genetic factors. Maternal diseases (e.g., diabetes mellitus), clinical drugs (e.g., valproic acid), and environmental factors (e.g., alcohol, pollutants) are among the non-genetic factors ascribed for rapid increases of birth defects [3,4]. Efforts to prevent birth defects have been made at the population level, for example, dietary vitamin supplementation. Individualized and defect type-focused practices require detection and diagnosis of embryonic malformations during early pregnancy.

Embryonic malformations

Structural defects are most commonly seen in the central nervous and cardiovascular systems (CNS and CVS). In the CNS, anomalies are present in the brain (e.g., exencephaly) and spinal cord (e.g., spina bifida) [5]. These abnormalities are formed due to failure in neural tube closure during the early embryogenesis, thus, referred to as neural tube defects (NTDs) [5]. The process of neural tube formation, namely neurulation, occurs between 18-26 days of post-conception in humans [6]. In the CVS, most common anomalies are associated with abnormal cardiac septation to produce septal defects [7]. These defects are also formed during the early cardiogenesis [7]. Therefore, any measures to prevent birth defects should be implemented before or during the so-called susceptible period of organogenesis, which is the early first trimester.

Diagnosis of embryonic/fetal anomalies

Detection of embryonic/fetal anomalies in pregnancies largely relies on ultrasonography [8]. With recent technological developments, imaging resolution and processing have been considerably improved [9]. Color Doppler sonography can measure fetal cardiac functions [10,11]. However, ultrasonography has limitations in detecting structural abnormalities in young embryos. Most abnormalities are only diagnosed in fetuses older than 12 weeks of gestation [9,12]. Very few anomalies can be recognized as young as 10 weeks of gestation [13]. By then, the damages in the fetuses are already irreversible.

Biomarkers for diagnosis of fetal anomalies

Searching for molecular biomarkers in maternal blood and amniotic fluid has been undertaken for decades. Because of the limited accessibility of the amniotic fluid, maternal blood is the major source of biomarkers.

One class of biomarkers, reflecting maternal physiological conditions, for example, oxidative stress markers (endogenous antioxidants and lipoperoxidation products), have been used to evaluate the risk of developing fetal abnormalities [14,15].

Protein biomarkers, including α-fetoprotein, human chorionic gonadotropin, maternal serum unconjugated estriol, and Inhibin-A, have also been characterized and used in diagnosis of fetal anomalies in pregnancies [16-19]. However, these biomarkers can only be used as supplementary indicators to ultrasound examinations because of their low sensitivity and reliability.

MicroRNAs as biomarkers for detection of embryonic malformations

Recently, nucleic acids (DNA and RNA), especially non-coding RNAs, have been found in human blood and urine [20-23]. Among them, microRNAs (miRNAs) have drawn much attention as potential biomarkers for diseases [22,24,25]. MI RNAs are 22 not single-stranded RNAs, present intracellularly and extracellularly [26-28]. Mi RNAs are

***Corresponding author:** Zhiyong Zhao, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD 21201, USA, Tel: (410) 706-8401; E-mail: zzhao@fpi.umaryland.edu

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initially synthesized as long primary miRNA (pri-miRNA) molecules by RNA polymerase II or III. Pri-miRNAs are processed by nuclear endoribo nuclease Drosha into ~70 nt hairpin structures, known as pre-miRNA. The pre-miRNAs are transported out of the nucleus by Exportin 5. In the cytoplasm, the pre-miRNAs are further processed by another RNase, Dicer, to become 22 not single-stranded miRNAs [29-31]. Within the cell, miRNAs, facilitated by a protein complex, known as RNA-induced silencing complex (RISC) containing argonaute (Ago) proteins, bind to messenger RNAs (mRNAs) via the Waltson-Crick complementary base-pairing to block translation or induce mRNA degradation [32-34].

MiRNAs are expressed in the developing embryo and have been demonstrated to play an important role in embryogenesis [35-37]. Dysregulation of miRNAs has been shown to be associated with human birth defects, e.g., Tetralogy of Fallot in the heart and Di George syndrome [38,39]. Spatial and temporal expression patterns of miRNAs in the developing primitive organs of the embryo, such as the neural fold and heart tube, imply that they are involved in malformations of the structures during the period of organogenesis susceptible to environmental insults [36,40-42]. Animal studies have shown that deficiency in miRNA biogenesis and processing results in abnormal embryogenesis. For example, embryos lacking the *dicer1* or *ago2* genes, or certain species of miRNA (knockouts) fail to develop beyond gastrulation or develop abnormalities in many organ systems, including NTDs and heart defects [43-47], resembling birth defects in humans.

The mechanisms by which miRNAs regulate development remain to be fully delineated. Available data have suggested that miRNAs target genes that regulate neurulation and cardiogenesis, including transcription factors and genes in growth factor signaling (e.g., the TGFβ and Wnt families) [36,40,48,49].

MiRNAs, are present in the amniotic fluid and potentially used to predict outcome of pregnancy [50,51]. However, invasive methods to collect amniotic fluid during gestation are not practical. MiRNAs are excreted from cells and transported into blood stream (Figure 1), and can be obtained from maternal blood samples collected during prenatal care [22,24]. MiRNAs are promising biomarkers for early detection of embryonic malformations in pregnancies, for the following reasons. First, miRNAs are very stable in body fluids [23]. Their initial appearance in the blood can indicate the early stages or even before the onset of diseases. Changes in their levels can reliably reflect the progression of diseases. Second, embryonic miRNAs can pass through the maternal-embryonic interface to be present in the maternal system [52,53]. Third, due to the characteristic of base-sequence, certain species of miRNA can provide unique signatures of embryonic conditions. Fourth, technologies to amplify and sequence miRNAs can detect tiny amounts of miRNAs for early diagnosis and provide sequence-specific information [54,55].

Pregnancy-related miRNAs have been isolated from maternal plasma [56-58]. Microarray, sequencing, and quantitative reverse transcription-coupled polymerase chain reaction assays revealed that the expression profiles of miRNAs are correlated with fetal malformations in the CNS and CVS [56,57]. These studies demonstrate the potential application of this approach to diagnosis of fetal anomalies.

More research is needed to validate the reliability and sensitivity of miRNA markers in detecting embryonic malformations [59]. For example, maternal blood samples are usually collected during the secondary and third trimesters [56,57]. However, embryonic malformations occur during the early first trimester [4,60]. MiRNAs expressed during early embryogenesis, the so-called susceptible period

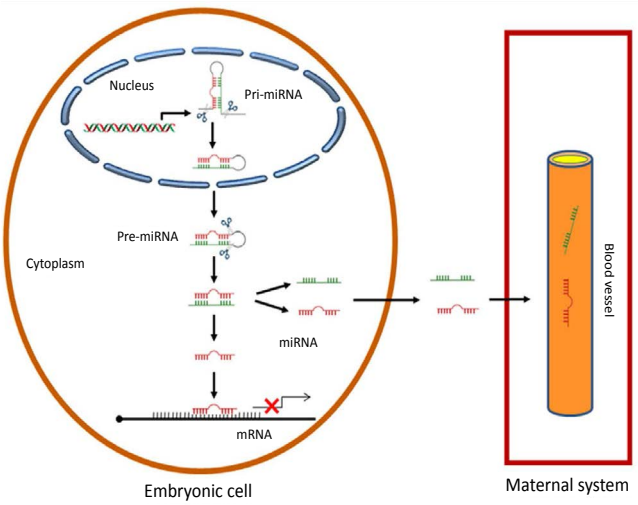

Figure 1: Diagrammatic illustration of embryonic miRNA biogenesis and secretion to maternal circulation. Transcription of miRNA genes generates pre-miRNAs in the nucleus of embryonic cell. pri-miRNAs are processed into pre-miRNAs and transported to the cytoplasm. Pre-miRNAs are cleaved into double-stranded small RNA, and further separated into single-stranded miRNAs. Mi RNAs are either involved in post-transcriptional regulation of mRNAs or secreted into extracellular space and transported into maternal circulation.

of organogenesis, may more reliably reflect the developmental conditions in dysmorphogenesis. Secondly, it is important to distinguish embryonic miRNAs from maternal miRNAs, as they represent different biological processes and may possess different sensitivity. With the recent technologies to amplify and sequence small RNAs, miRNAs can serve as biomarkers to detect risks and early aberrant developmental events in pregnancy, making early diagnosis and individualized birth defect prevention a reality.

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