Noninvasive nucleic acid–based approaches to monitor placental health and predict pregnancy-related complications

Irina Manokhina, MD, PhD; Samantha L. Wilson, BSc; Wendy P. Robinson, PhD

During pregnancy, the placenta releases a variety of nucleic acids (including deoxyribonucleic acid, messenger ribonucleic acid, or microribonucleic acids) either as a result of cell turnover or as an active messaging system between the placenta and cells in the maternal body. The profile of released nucleic acids changes with the gestational age and has been associated with maternal and fetal parameters. It also can directly reflect pathological changes in the placenta. Nucleic acids may therefore provide a rich source of novel biomarkers for the prediction of pregnancy complications. However, their utility in the clinical setting depends, first, on overcoming some technical considerations in their quantification, and, second, on developing a better understanding of the factors that influence their function and abundance.

Key words: cell-free deoxyribonucleic acid, cell-free messenger ribonucleic acid, microribonucleic acid, pregnancy complications, screening

The placenta is responsible for the growth and development of the fetus in multiple ways. It transfers gases and nutrients from maternal to fetal blood, provides immune protection, and is generally responsible for regulating the fetal environment. Hence, the assessment of placental health is an essential part of obstetrical care. Traditional clinical approaches to assess placental function include the following: (1) evaluation of morphology and placement with ultrasound, (2) umbilical and uterine artery Doppler ultrasound to measure blood flow, and (3) measurement of maternal serum proteins that are mainly excreted by the placenta and can reflect altered placental function.[1,2]

In addition to placental proteins, placental-derived nucleic acids (deoxyribonucleic acid [DNA] and ribonucleic acid [RNA]) can enter the mother's bloodstream and other body fluids,[3-6] providing a rich source of material that can be measured to monitor placental function and allow early diagnosis of pregnancy complications.[7,8]

From the Child and Family Research Institute (all authors), and Department of Medical Genetics, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada (all authors).

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Corresponding author: Wendy P. Robinson, PhD. wrobinson@cfri.ca

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The analysis of cell-free fetal DNA in maternal blood has proved valuable for the noninvasive prenatal diagnosis of genetic mutations and chromosomal abnormalities in the fetus. An additional application is to quantify its level as a biomarker of abnormal placentation, similar to measuring of maternal serum alpha-fetoprotein (AFP) and beta-human chorionic gonadotropin (β-hCG) levels. Furthermore, quantification of messenger RNA (mRNA) encoding for proteins found in maternal serum, may more directly reflect placental gene expression. Non-coding microRNA (miRNA) is a subclass of regulatory molecules that have been described as a new type of hormones because of their mode of synthesis, delivery, and effects.[9,10]

In this review, we will discuss how nucleic acids are released into maternal blood, the approaches used to quantify them, and their application as biomarkers to assess placental health.

Placental signature in the maternal circulation

During pregnancy, nucleic acids derived from the placenta can be released into the maternal circulation as a part of normal extravillous trophoblast invasion, release of deported trophoblast knots, breakdown of apoptotic/necrotic cells and blebbing of microvesicles from the trophoblast membrane, and active cellular communication systems involving microvesicles, nanovesicles/exosomes, and subcellular fragments[11,12] (Figure).

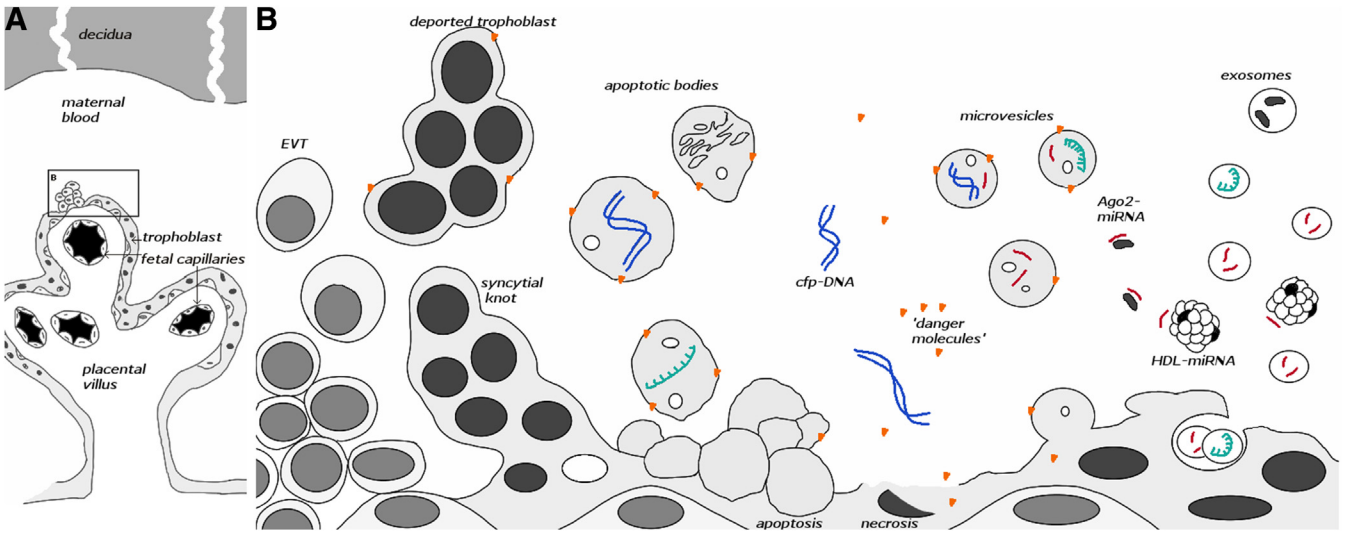
Extravillous trophoblasts

These are cytotrophoblast-derived mononuclear cells that are programmed to invade the maternal uterus and migrate into and remodel the maternal vessels.[13] Because invasion of the maternal uterine vasculature is a normal property of extravillous trophoblast cells, it would be expected to find them in maternal circulation. However, intact fetal- or placental-derived cells are challenging to detect with typically a ratio of less than 1 fetal/placental cell to 100,000 maternal cells.[14]

Multinuclear trophoblast fragments

A variety of multinuclear syncytial structures described as deported trophoblasts and thought to be derived from syncytial trophoblast knots are present in the maternal circulation.[11] Deported trophoblast structures are found in uterine vein blood of normal pregnancies, with higher levels in women experiencing preeclampsia (PE).[11] However,

FIGURE
Placental debris in relation to nucleic acids transfer



A, Placental villus with zoom-in area. **B**, Zoom-in area (cytotrophoblast, syncytiotrophoblast, and their 'debris'). *Blue* indicates DNA, *green* indicates mRNA, and *red* indicates miRNA. The figure is not to scale.

Ago2, protein argonaute-2; *DNA*, deoxyribonucleic acid; *EVT*, extravillous trophoblast; *HDL*, high density lipoproteins; *miRNA*, microribonucleic acid; *mRNA*, messenger ribonucleic acid.

Manokhina. Nucleic acid biomarkers of pregnancy complications. Am J Obstet Gynecol 2015.

these trophoblasts are rarely found in the maternal peripheral circulation, being mostly trapped in the lung capillary system because of their large size, with a subsequent lysis and release of nucleic acids into circulation.[15,16] There is evidence that deported trophoblast knots may be transcriptionally active in the maternal bloodstream and synthesize a significant proportion of placental mRNA and proteins.[17,18]

Microvesicles (anuclear bodies)

Apoptosis and necrosis are thought to be the major mechanisms of release of placental nucleic acids into the maternal circulation.[19] The associated particles can be either apoptotic bodies, which arise due to cell sequestration and may contain organelles as well as nucleic acids, or different types of syncytiotrophoblast microvesicles (STBMs), which appear because of the blebbing of the plasmalemma as a response to either apoptotic or activating signals.[11] The pathophysiological effect of circulating STBMs is associated with several nonspecific molecules that have proinflammatory and disrupting effects on the maternal endothelium only after being released from the cell (so-called danger molecules, ie, tissue factor, fibronectin, or heat shock protein).[11,20]

The shedding of STBMs may be increased in response to hypoxia and reoxygenation stress that is associated with shallow placentation and leads to a cascade of maternal immunological responses that contribute to endothelial damage and maternal hypertension.[21] Necrosis likely coexists with apoptotic processes but occurs more frequently when coupled with a stressful environment.[22] Necrosis, as a direct cell destruction process, is also thought to cause the release of proinflammatory danger molecules as well as nonassociated free forms of cell-free DNA or mRNA.[21,23]

Exosomes/nanovesicles (active transport vectors)

Another way nucleic acids are released into the maternal blood is through mechanisms of active cell communication, whereby selected molecules undergo specific packaging and are then actively secreted into the maternal bloodstream in which they can be incorporated into target cells by endocytosis.[12] This mainly involves microRNA (miRNA) and mRNA molecules, and the transport mechanisms involve both vesicles (exosomes and microvesicles) and complexes of subcellular components (argonaute-2 proteins, high-density lipoproteins).[12,24]

The concentration of exosomes is increased (>50-fold) in the plasma of pregnant women compared with nonpregnant women and correlates with physiological and pathological parameters (eg, their frequency is correlated positively with placental size and negatively with mean uterine artery pulsatility index, which is increased in intrauterine growth restriction [IUGR] and PE).[25] miRNAs associated with proteins or lipoprotein complexes can be delivered to other cells to directly alter gene expression.[26,27] The physiology of the release of different nucleic acids and the rates of their free and associated forms are not completely understood.

Analysis approaches

Nucleic acid fragments released from the placenta provide a rich source of novel