Review

Is there a sex of the placenta?

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Proceedings

Abstract

The placenta has traditionally been considered as an asexual organ. Thus, most of the studies focusing on the placenta have not taken the sex of the embryo into account.

However, as trophoblast cells originate from the embryo, they reflect fetal sex as either XX or XY, allowing for possible sex differences in placental biochemistry, function, and signaling.

The placenta is a temporary organ performing the functions of many adult organs for the growing fetus. The placenta plays a key role in fetal growth and development, it is designed for exchange of oxygen, nutrients, antibodies, hormonal compounds and waste products between the mother and fetus and may carry significant information about the pregnancy. The placenta is considered also a major endocrine organ being responsible for synthesizing vast quantities of hormones and cytokines that have important effects on both maternal and fetal physiology.

The investigation of placenta and its functions helps to identify molecular mechanisms that have both early- and long-term effects on health of the fetus.

Gender differences were observed in the placenta at multiple levels: epigenetic modifications of DNA, gene expression, protein expression and immune function.

Keywords

Placenta, sex, epigenetics, immune function, review, pregnancy, outcome.

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Introduction

The placenta has traditionally been considered as an asexual organ. Thus, most of the studies focusing on the placenta have not taken the sex of the embryo into account.

However, as trophoblast cells originate from the embryo, they reflect fetal sex as either XX or XY, allowing for possible sex differences in placental biochemistry, function, and signaling.

Ishikawa et al. [1] have clearly established an effect of sex chromosome "dosage", independent of androgen, on placental size in mice, with XX placentas being significantly smaller than XY placentas. Although the presence of two X chromosome rather than one leads to a decrease in placental size, the basic mechanism is still to be clarified.

Moreover, all males cells possess a single X chromosome of maternal origin and a Y chromosome of paternal origin while female cells consist of two population, one with inactive maternally inherited X and the other with inactive paternally inherited X. Further, X chromosomes in the placenta could be reactivated or inactivated in response to intrauterine conditions [2]. This plasticity in X-inactivation in the placenta may be an important contributor to sex-differences in response to environmental perturbations during gestation, whereby females may be buffered from detrimental conditions to a greater degree than males due to increased expression of important X-linked genes.

Many studies [3-8] have observed gender specific differences in fetal growth and fetal and neonatal morbidity and mortality.

Mortality in both the number of stillbirths and neonatal deaths are greater among males than females. Significant effects of fetal gender on pregnancy outcome and the development of pregnancy-related complications were observed more frequently among male neonates with regard to preterm birth, pre-eclampsia, gestational diabetes mellitus, nuchal cord and true umbilical cord knots.

Other gender differences are related to birthweight range, generally larger in males than females while females are more likely growth reduced. More males were delivered than females rather than an expected 1:1 ratio.

To safeguard the fetus, the placenta must be able to detect changes and rapidly adapt to mutable environmental conditions in utero. The placenta of one sex over the other might possess greater capacity to respond to such environmental changes. An inadequate response of the placenta to these alterations could contribute to detrimental developmental origins of adults health and disease (DOHaD) effects. Gender differences occur in many diseases, including metabolic diseases, hypertension, cardiovascular disease, psychiatric and neurological disorders and cancer. For example, men are more predisposed to cardiovascular disease while women are more predisposed to obesity. Although several factors contribute to the risk of adult cardiovascular disease, including smoking and elevated body mass index, many epidemiological studies suggest that there is a "fetal origin" that predisposes adults to these disorders [9].

Placental pathologies linked to male or female sex were observed amongst high-risk pregnancies with severe placental dysfunction.

Velamentous umbilical cord insertions and chronic deciduitis were more frequent in males, while higher rate of villous infarction in females. Generally, complicated pregnancy with male than female fetus are more frequently associated with adult hypertension, unfavorable lipid profiles as young adults, higher death rate of ischemic heart disease, increased risk of stroke, higher incidence of coronary heart disease, subclinical atherosclerosis and myocardial infarction.

Considering the potential effect of the placenta on the health of the offspring, many studies have been focusing on this organ.

The placenta performs the functions of many adult organs for the growing fetus. The placenta is essential in fetal growth and development, it is designed for exchange of oxygen, nutrients, antibodies, hormonal compounds and waste products between the mother and fetus. The placenta is considered also an important endocrine organ since it synthesizes many hormones and

cytokines that have central effects on both maternal and fetal physiology.

Therefore, the investigation of placenta and its functions could help to discover molecular mechanisms that have both early- and long-term effects on health of the fetus.

Gender differences were observed in the placenta at multiple levels: epigenetic modifications of DNA, gene expression, protein expression and immune function.

Epigenetic modifications of DNA

Epigenetic modifications of DNA occur without any alteration in the underlying DNA sequence and can control whether a gene is turned on or off and how much of a specific message is transmitted.

Every cell in the body has the same DNA sequence but different genes are turned on or off to make different tissues, such as skin, kidney or liver.

The placenta is influenced by numerous environmental factors including nutrients and tissue oxygenation, which may modify epigenetic marks and gene expression within the placenta and consequently placental development and function.

The resulting alterations in epigenetic marks may alter cell fate decisions, the subsequent growth and development of tissues and organs, and consequently be responsible, in a sex-specific manner, for inadequate responses to later challenges such as a hyperglicemic environment.

The sex of the placenta and the environment affects its epigenomes, and therefore the epigenomes of the developing fetus. In many adult tissues, such as gonads, brain, and liver, the expression of numerous genes is regulated in a sex-specific manner [10, 11].

At present, the three major epigenetic factors are post-translational histone modifications, DNA methylation, and small noncoding RNAs, such as microRNAs (miRNAs). All of these processes are closely connected. For example, changes in histone marks, such as acetylation or methylation, are necessary for DNA methylation and demethylation to occur [12]. DNA methylation can regulate miRNA expression and vice versa [13, 14], and miRNA frequently target and regulate levels of the histone-modifying enzymes deacetylases and methyltransferases [15-18]. The exact location and combination of these modifications determines small- and large-scale chromatin conformational changes [19].

Most studies on DOHaD have reported sex-dependent effects, but very few have delineated the sex-specific epigenetic mechanisms involved, and especially in the placenta. In a recent review paper [20], Novakovic and Saffery suggested that DNA methylation profiling underlines the unique nature of the human placental epigenome for genomic imprinting and placenta-specific gene-associated methylation. Placental cell types present a pattern of genome methylation which is significantly different from that in somatic tissues, with a low grade methylation at some, but not all, repetitive elements.

In the human placenta, increased methylation of the leptin receptor gene is associated with increased lethargy and hypotonicity in male, but not female newborns [21], supporting not only a connection between epigenetic regulation of the leptin receptor and the relay of important information to the fetal brain, but also a sex specificity in these outcomes.

In mice, female placental tissue has higher levels of global DNA methylation compared to male placentas [22], which may provide females additional protection from dynamic changes in gene expression resulting from environmental insults. In addition, tighter regulation of gene expression in females provides a plausible mechanism by which males are preferentially affected by exogenous insults, such as maternal stress during gestation.

MiRNAs are highly conserved, regulatory molecules that have an important role in the post-transcriptional regulation of target gene expression by promoting mRNA instability or translational inhibition [23]. MiRNAs are expressed in placenta and alterations in their expression have been described in association with exposure to xenobiotics [24], cigarette smoking [25] or with adverse pregnancy outcomes including preeclampsia [26] and growth restriction [27]. Moreover, they may play a role in regulating sex specific gene expression.

One seminal study, that set the *de novo* landscape of miRNA-regulation in cells of the trophoblast lineage, was published in 2012 by Morales-Prieto et al. [28]. In this study, the authors screened 762 human miRNAs for their expression level in term and first trimester cytotrophoblasts. One of the major outcomes of this work was the identification of clusters of placenta-specific miRNAs (C19MC, 54 miRNAs on chromosome 19, C14MC, 34 miRNAs on chromosome 14, and another minor cluster on chromosome 19). Placenta-specific miRNAs epigenetically regulate

the expression of gene sets associated with both adaptive and innate immune responses throughout pregnancy, while miRNAs controlling oncogenic, angiogenic and anti-apoptotic genes appear dominant during the first trimester, miRNAs promoting cell differentiation are highly expressed in late pregnancy [29].

Moreover, these miRNAs allow placenta to respond to adverse maternal environment such as obesity [30] or pre-eclampsia [31] in a fetal sex-specific manner. In placentas of female fetuses but not in those of males, an activation of signaling from inflammation via NFϰB1 and miR-210 leading to mitochondrial dysfunction was observed. These data suggested that primary trophoblasts derived from placentas of female fetuses showed higher sensitivity to inflammatory stress compared with placentas of males. Moreover, they evidenced the essential role of maternal inflammatory status in regulation of placental mitochondrial metabolism and identify miR-210 as a central component of this fetal sex-biased metabolic regulatory mechanism.

Given the importance of genomic imprinting in the placenta, this provides new clues for further investigations of sexual dimorphism in the placenta. This sex difference in epigenome likely accounts for aspects of the reported sex differences in gene and protein expression in the placenta.

Gene and protein expression

Global gene changes in the human placenta have been analyzed by Sood et al. [32]. The study clearly defined sex specific differences in placental gene expression not limited to just X and Y linked genes but also autosomal genes related to immune pathways including JAK1, IL2RB, Clusterin, LTBP, CXCL1 and IL1RL1 and TNF receptor expressed at higher levels in female placentae than male placentae. The differences in immune gene expression may contribute to gender differences in the fetal response to infection or inflammation.

Analysis of genes involved in amino acid transport and metabolism identified gender differences both in average placental gene expression between males and females and in the relationships between placental gene expression and maternal factors, suggesting a higher global transcriptional level in females and greater protein metabolism levels in males [33].

Preliminary microarray studies have identified 59 gene changes in the placentae of females and only six gene changes in placentae of males in pregnancies complicated by asthma. These data show that female placentae institute multiple gene alterations that interconnect with numerous signalling networks to cope with the presence of maternal asthma; some of these networks may contribute to reduced growth. The minimal placental gene alterations of the male placentae may allow the male fetus to continue growing in an adverse environment.

Immune function

Sex specific differences in immune function in the placenta regard expression of cytokine mRNA, including TNF-a, IL-1b, IL-6, IL-5 and IL-8, which vary with asthma severity and fetal sex. All cytokines were observed to be increased in female placentae of pregnancies complicated by asthma if compared to female control placenta. The male fetus has no significant alterations in placental cytokine mRNA expression in the presence of maternal asthma.

Sex differences of the fetal-placental immune system have been also investigated in relation to preterm delivery. According to Ghidini and Salafia [34], histological examination of placentae of males delivered less that 32 weeks gestation revealed more severe lesions of chronic inflammation than placentae from matched females. The sites of chronic inflammation were areas of interaction between interstitial trophoblast and maternal decidua rather than within placental villi or membranes: these data suggest that the maternal immune system induces an inflammatory response in the placenta via the decidua. Male neonates were more likely to have an infected placenta than female neonates with greater decidual lymphoplasmacytic cell infiltration.

More recently Yeganegi et al. [35] have reported that male placentae have higher toll-like receptor-4 (TLR-4) expression and a more enhanced endotoxin induced tumor necrosis factor (TNF)-α response relative to placentae from females. Since a greater population of placental macrophages has been identified in males relative to females of normal pregnancies, the enhanced TNF-α response may be derived from a sex difference in immune cell populations. They suggest that this sex difference in cytokine production may contribute to the increased incidence of preterm delivery in males.

These data demonstrate that placental immune function is at least partially sex specific and

suggests that the placenta responds to maternal inflammatory status in a sex specific manner.

Moreover, these findings can help for understanding the impact of maternal viral, bacterial and parasitic infections during pregnancy such as HIV, pneumonia and malaria on fetal growth and survival. It also has relevance to understanding the impact of maternal inflammatory states that can complicate pregnancy including obesity, rheumatoid arthritis, asthma and Crohn's disease. Preeclampsia has been identified as an inflammatory state and may also influence placental immune function in a sex specific manner.

Since the placental immune system plays a role in regulating apoptosis, prostaglandin synthesis, vascular permeability and programming of the fetal immune system, it is possible that all these mechanisms are sexually dimorphic.

Conclusions

Female and male placentas have different strategies to optimize health: actually, the two sexes present different optimal transcriptomes that may affect fetal growth and later health or disease.

The male strategy for responding to an adverse maternal environment is a minimalist approach: few genes, proteins or functional changes are involved in the placenta, which ultimately ensures continued growth in a less than optimal maternal environment.

This specific male response is associated with a greater risk of intrauterine growth restriction, preterm delivery or death *in utero* if another adverse event occurs during the pregnancy.

The female placenta responds to an adverse maternal environment with multiple placental gene and protein changes that result in a decrease in growth without growth restriction (> 10th centile).

Thus, female adjustments in placental function and growth ensure survival in the presence of another adverse event which may further compromise nutrient or oxygen supply.

The placenta could be an ideal system to study fetal stresses, starvation, endocrine disruption and obesity-prone diets or lifestyles, in a sex-specific manner. In conclusion, to use the placenta as an indicator of what occurred *in utero*, it is important to understand how, in addition to sex-specific differences in the endocrine and immune systems, sex-specific genetic architecture also affects placental growth and specific placental functions, either in normal conditions or in severe placental pathologies that could bring to adverse pregnancy outcomes.

Declaration of interest

The Authors declare that there is no conflict of interest.

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