Epigenetics in placental development

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Abstract

Placenta, by virtue of its critical structural and functional roles, is a key link in the chain of events that lead to intrauterine programming of adult health. The critical role of epigenetic regulation, the mitotically and meiotically heritable control of gene expression not related to deoxyribonucleic acid sequence, during development is becoming increasingly appreciated. In this review, we concentrate specifically on the intersection between transcriptional, epigenetic, and physiological factors in specifying placental development. Additionally, fetal programming mechanisms depend on the interplay of transcriptional and epigenetic regulators with environmental cues to induce alterations that manifest as disease susceptibility in adults. We tried to summarize the current knowledge in the field of epigenetics in relation to placental development and function.

Keywords: *epigenetic mechanisms, gene expression, placenta*

Abbreviated words: *DNA= deoxyribonucleic acid; RNA= ribonucleic acid; miRNA= microRNA; DMRs= differentially methylated regions; iCTBs= invasive cytotrophoblasts; MBC= maternal blood cells; ESCs= embryonic stem cells; ADP= adenosine diphosphate; GTD= gestational trophoblastic disease; LOI= loss of imprinting; HATs= histone acetyltransferases; HDACs= histone deacetylases*

1. Introduction

The placenta is a complex organ. Short lived by design, its brief existence enables the mammalian fetus to survive within the confines of the intrauterine environment. The diversity of functions performed by the placenta is impressive, ranging from anchoring the concepts and preventing its rejection by the maternal immune system to enabling the transport of nutrients and wastes between the mother and the fetus[1]. As with all organs, it performs these functions via multiple specialized cell types derived from lineage-committed precursors that either proliferate or differentiate. This process depends on a coordinated interaction among genetic, epigenetic, and physiological cues that are differentially interpreted as a function of gestational age[2,3].

Epigenetics, an area of genomics that is attracting intense interest, can be loosely defined as the study of cellular "traits" that influence biological phenotype in a fashion that is not dependent on the underlying primary deoxyribonucleic acid (DNA) sequence[1]. Of particular significance is that epigenetic change in genome function can result in altered phenotypic states that are not only sustained in the short term, but may be heritable in a mitotic and meiotic fashion[4,5].

Gene environment interactions are centrally involved in our susceptibility to disease and these influences are likely to be mediated to a large degree via epigenetic regulatory phenomena[6]. Epigenetic mechanisms form the foundation of a process termed programming, in which a cellular memory is imposed upon the progeny of lineage-committed precursors to ensure both the acquisition and maintenance of a terminally differentiated state.

The four main epigenetic mechanisms are: DNA methylation, imprinting, histone modification, and small ribonucleic acid (RNA)-mediated control, specifically (micro) miRNAs. DNA methylation is carried out by one of a variety of DNA methyltransferases responsible for adding a methyl group to cytosine residues in cytosine/guanine-rich regions of DNA (called "CpG islands"); when a given stretch of cytosines in a CpG islands located in the promoter region of a gene is methylated, that gene will be effectively silenced by methylation. The phenomenon of genomic imprinting is the parent-of-origin allele specific expression of genes; genes controlled through imprinting are often located and regulated coordinately in clusters; imprinted genes are theorized to be controlled at differentially methylated regions (DMRs) by DNA methylation; both non-coding RNAs and changes in DNA methylation at sites in DMRs are responsible for the regulation of the imprint and although DNA methylation is involved, it does not function in a manner similar to that seen in promoter regions[7].

2. Epigenetics and Intrauterine Development

One setting in which epigenetics is likely to have a profound influence on biological phenotype is during intrauterine development. In this context, there is a defined and critical window during which balanced homeostasis is essential for normal fetal growth and development. Because of its central role in guiding fetal development and acting as the gatekeeper of maternal environmental exposure, the placenta responds to and is potentially marked in an epigenetic context by environmental insults which suggests that the placental epigenome might serve not only as a record of *in utero*

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exposure, but also as a mediator and/or modulator of disease pathogenesis[7,8].

The placenta contains both maternal and fetal structures. While placental cells (trophoblasts) initially proliferate much more rapidly than the embryo following implantation[9], formation of the maternal-fetal interface and subsequent oxygen and nutrient transport result in a 40-fold increase in the fetus weight ratio in humans at term[10]. This may lead to a massive increase in placental transport capacity due to a diverse set of developmental processes, including branching and nonbranching angiogenesis, branching morphogenesis, and trophoblast differentiation into several cell types, including invasive cytotrophoblasts (iCTBs) and multinucleated syncytiotrophoblasts. iCTB invasion anchor the human conceptus to the uterus, and the endovascular component of this process enables remodeling of maternal spiral arterioles, thereby establishing blood flow to the placenta. A subset of iCTBs breaches spiral arterioles and differentiates into an endovascular subtype that replaces the resident maternal endothelium and intercalates within the smooth muscle walls of the vessels. In a fascinating transdifferentiation process, human iCTBs with a primarily epithelial phenotype acquire vascular/endothelial characteristics. The component steps include downregulation of integrin α6β4 and E-cadherin and upregulation of integrins αVβ3 and α1β1, VE-cadherin, vascular cell adhesion molecule-1, and platelet endothelial cell adhesion molecule-1. Additionally, iCTBs produce a number of proteins that are involved in extracellular matrix degradation[11].

3. Placental Epigenome and Genetic Factors

Tianjiao and colleagues[12] have carried out a detailed structural and functional analysis of the placental epigenome at its maternal interface performing genome wide analysis of DNA methylation in samples of chorionic villus and maternal blood cells (MBC) using both commercially available and custom designed microarrays. They discovered that other genomes are significantly more hypomethylated than their MBC counterparts and they also identified unique patterns of DNA methylation associated with distinct genomic structures such as gene bodies, promoters and CpG islands. They established both direct and inverse relationships between DNA methylation levels and gene expression levels in gene bodies and promoters respectively. Furthermore, they found that these relationships were significantly associated with CpG content, concluding that the early gestational placental DNA methylome is highly organized and is significantly and globally associated with transcription.

The earliest differentiation event in the mammalian embryo has occurred by the blastocyst stage where the inner cell mass separates from the outer layer, the trophectoderm. While some genetic factors have been identified that are crucial for the establishment of early cell lineage identity, little molecular information exist about the epigenetic contribution to their differentiation and/or maintenance. Hemberger[13] investigated whether epigenetic factors influence the fate of early cell lineages by determining the trans-differentiation potential of embryonic stem cells (ESCs) into derivatives of the trophoblast lineage. The author had previously reported also that poly(adenosine diphosphate (ADP)-ribosyl)ation was one modification required for maintaining ESC identity because trophoblast derivatives arose more frequently from ESCs deficient in the poly(ADP-ribosyl)ating enzyme, Parp1. Dissecting the process of how poly(ADP-ribosyl)ation contributes to maintaining ESC identity may be challenging, however, because 18 genes are known to exhibit poly(ADP-ribosyl)ating activity with potential for functional redundancy[13]. However, there is only one gene, poly (ADP-ribose)glycohydrolase (Parg), that catalyses the hydrolysis of poly(ADP-ribose) leading to its degradation into free ADP-ribose moieties.

Imprinted genes represent a small number of genes in the mammalian genome whose expression depends on whether they come from mother or father. Imprinted genes are marked in germ cells providing a heritable 'memory' that reveals their parental origin and results in their subsequent allelic activity or repression. DNA methylation and histone modifications are the best-characterized marks with DNA methylation being known to be essential for germline imprinting[14]. Constancia et al.[15] gave an overview of the expression and function of imprinted genes in the placenta. Of the 54 protein-coding imprinted genes identified to date in the mouse, no placental information is available for 22 of them. In a census of the remaining 32, the authors indicated that 10 are imprinted in the placenta only and, interestingly, these are all expressed from the maternally inherited chromosome. Twenty out of 32 are imprinted in both the placenta and the fetus and include imprinted genes expressed from either the maternally, or the paternally inherited chromosome[15]. Ferguson-Smith and Surami[16] studied the epigenetic mechanisms governing the regulation of imprinted genes leading to a consideration of the mechanism of imprinting from an evolutionary perspective. All imprinted gene clusters to date are regulated by methylation differences on the two parental chromosomes acquired in the male and female germlines[14]. For the majority of clusters methylation is acquired during oogenesis and interestingly many of these clusters show tissue-specific imprinting, often in the placenta. Imprinting has been theorized to be one of the mechanisms involved in the so-called "parent conflict" theory[16]. This theory suggests that paternally expressed genes strongly favor using maternal resources to benefit offspring, while maternally expressed genes attempt to preserve such

maternal resources and thus, are in direct conflict with one another[16]. In such a way, one could argue that paternally expressed (and maternally imprinted) genes would work to foster the growth of offspring, while maternally expressed (and paternally imprinted) genes would function to better ensure that each offspring has approximately the same access to maternal resources as its siblings[17].

4. DNA Binding Protein and Transcription Factors

In the mid-1980s, researchers demonstrated the effects of site-specific DNA methylation on the binding ability of DNA-binding protein in human placenta[18], contributing greatly to understanding the effects of DNA methylation patterns on the recruitment of DNA binding elements and subsequent effects on transcription[19]. Some researchers have focused on links between aberrant methylation patterns of placental gene promoters and disease progression. Work by Zhang and colleagues has further demonstrated that the Oct4 transcription factor whose hypermethylation is associated with downregulation of gene expression and differentiation of trophectoderm cell lineage is downregulated by increased methylation in normal placenta and in gestational trophoblastic disease (GTD), an epigenetic regulatory mechanism which may prove important in the development and progression of GTD[20]. In work to better characterize the genetic and epigenetic factors underlying the onset and progression of preeclampsia, Chelbi and colleagues suggested that aberrant methylation patterns may be a typical mechanism ultimately leading to preeclampsia[21].

Novakovic et al. have investigated placenta-specific methylation patterns which maximize the bioavailability of essential vitamins, specifically vitamin D, at the fetomaternal interface[22] and Park et al. have demonstrated the association of folate and homocysteine levels and DNA methylation levels in the human placenta[23].

Lambertini et al. have utilized techniques to measure loss of imprinting (LOI) in genes in human placentas and have concluded that not only LOI is common in human placentas, but may also serve as a key biomarker for epigenetics affected by prenatal conditions or environment[24].

Other researchers have investigated the effects of acetylation mediated by histone acetyltransferases (HATs) and the expression of important genes which play a role in the mediation of trophoblastic fusion. Chuang et al.[25] investigated key histone deacetylases (HDACs) that are involved in the deacetylation of the human GCMa transcription factor which plays a role in regulating syncytin, a placental protein that mediates trophoblastic fusion. Their data gave support to the theory that trophoblastic fusion in placental morphogenesis is largely dependent on the proper regulation of GCMa by HAT and HDAC.

Varying oxygen levels have effects on the genetic and epigenetic control mechanisms involved in placental growth and cell survival - and ultimately, on the health and survival of the developing fetus. Donker and colleagues[26] analyzed the relationships between expression of Argonaute 2, an important RNAi enzyme, and other miRNA in trophoblasts and in environments with varying oxygen level. Their data showed that not only is the miRNA processing machinery present and functional in human trophoblasts, but that varying expression of miR-93 and miR-424 is associated with different levels of oxygen. Such data may prove especially helpful in determining whether particular placental abnormalities, and ultimately, fetal abnormalities may be associated with aberrant levels of oxygen at particular critical windows of development.

Some studies investigated the aberrant expression of miRNA in the placenta. MiRNA base-pair to the 3′-untranslated region of target messenger RNA and effectively silence gene expression by a mechanism of either translational repression or direct messenger RNA degradation. Du and colleagues[27] have shown that partial complementarity of a miRNA to an messenger RNA target may result in effective repression of translation; therefore, a single miRNA can regulate a vast number of genes. Through this mechanism of post-transcriptional gene regulation, miRNA have been shown to regulate a number of key cellular functions including migration, invasion, growth and death. MiRNA exhibit tissue-specific expression and function and have been shown to be expressed in the placenta in addition to a variety of other tissues[28]. Alterations to placental miRNA expression have been associated with *in utero* exposures and adverse pregnancy outcomes[29].

Maspin, known as Serpin B5, a tumour suppressor gene, has been shown to play a role in the regulation of cell motility, invasion, apoptosis and angiogenesis. Some studies have reported the potential role of epigenetic mechanisms in regulating the expression of maspin in various cancer cell lines (hypermethylation of the maspin promoter in seven of nine breast cancer cell lines compared with normal human mammary cells). Maspin is differentially expressed in the human placenta. Decreased expression of maspin in the first trimester corresponds with the period of maximum trophoblast invasion, suggesting a role in cell invasion and motility. Dokras and colleagues investigated the role of epigenetic alterations in the regulation of maspin expression in the placenta and they found that maspin expression in the human placenta is regulated by changes in histone tail modifications[30].

5. Conclusion Remarks and Future Outlook

Epigenetic regulation of the placenta evolves during preimplantation development and further gestation. Epigenetic marks, like DNA methylation, histone

modifications and non-coding RNAs, affect gene expression patterns. These expression patterns, including the important parent-of-origin-dependent gene expression resulting from genomic imprinting, play a pivotal role in proper fetal and placental development. Disturbed placental epigenetics has been demonstrated in cases of intrauterine growth retardation and small for gestational age, and also appears to be involved in the pathogenesis of pre-eclampsia and GTD. Several environmental effects have been investigated so far, e.g. ethanol, oxygen tension as well as the effect of several aspects of assisted reproduction technologies on placental epigenetics. These results provide a unique insight into the structural and regulatory characteristics of the placental epigenome at its maternal interface and will drive future analyses of the role of placental dysfunction in gestational disease[24].

The data collected also further the hypothesis that the intrauterine environment acting through epigenetic alteration of the placenta is a key mechanism to explain the developmental origins of health and disease and suggest that enhanced examinations of the importance of placental epigenetic variation in health outcomes should be undertaken.

Epigenetic alterations may aid in disease diagnosis and prognosis as well as in targeting new treatment and prevention strategies. Genomic imprinting may play a critical role in placental biology, as the control of allelic expression is exaggerated in the placenta and alterations to these imprints have been linked to severe placenta pathologies[24].

Several studies have yield data demonstrating that miRNA expression is tissue-specific and that several miRNAs are expressed in the human placenta and much interest has been generated for investigating the involvement of miRNA in placental gene regulation and the possible utility of discovering placental miRNA, which can serve as clinical biomarkers of exposure or disease[28].

Examining epigenetic alterations in the placenta will prove especially important in the search for biomarkers of exposure, pathology, and disease risk and can provide critical insights into the biology of development and pathogenesis of disease. Studies in both animals and humans have made it increasingly clear that proper epigenetic regulation of both imprinted and non-imprinted genes is important in placental development. Its disturbance, which can be caused by various environmental factors, can lead to abnormal placental development and function with possible consequences for maternal morbidity, fetal development and disease susceptibility in later life.

Important advances in placental epigenetics continue to elucidate a better of understanding of the epigenetic regulatory mechanisms of in the placenta. Knowledge of such epigenetic mechanisms may be useful in identifying novel biomarkers for exposure, burden, or risk for disease. ■

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