Epigenetic regulation of human placental function and pregnancy outcome: considerations for causal inference

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Epigenetic mechanisms, often defined as regulating gene activity independently of underlying DNA sequence, are crucial for healthy development. The sum total of epigenetic marks within a cell or tissue (the epigenome) is sensitive to environmental influence, and disruption of the epigenome in utero has been associated with adverse pregnancy outcomes. Not surprisingly, given its multifaceted functions and important role in regulating pregnancy outcome, the placenta shows unique epigenetic features. Interestingly however, many of these are only otherwise seen in human malignancy (the pseudomalignant placental epigenome). Epigenetic variation in the placenta is now emerging as a candidate mediator of environmental influence on placental functioning and a key regulator of pregnancy outcome. However, replication of findings is generally lacking, most likely due to small sample sizes and a lack of standardization of analytical approaches. Defining DNA methylation "signatures" in the placenta associated with maternal and fetal outcomes offers tremendous potential to improve pregnancy outcomes, but care must be taken in interpretation of findings. Future placental epigenetic research would do well to address the issues present in epigenetic epidemiology more generally, including careful consideration of sample size, potentially confounding factors, issues of tissue heterogeneity, reverse causation, and the role of genetics in modulating epigenetic profile. The importance of animal or in vitro models in establishing a functional role of epigenetic variation identified in human beings, which is key to establishing causation, should not be underestimated.

Key words: confounding, development, Developmental Origins of Health and Disease, DNA methylation, epigenetics, epigenome, fetal programming, gestational age, gestational diabetes, intrauterine growth restriction, placenta, preeclampsia, pseudomalignant, reverse causation, trophoblast

Epigenetics is most commonly defined as "the heritable regulation of gene activity in the absence of change to the underlying DNA sequence."[1] However, this definition offers a very gene-centric view, disregarding important cellular functions such as DNA replication, centromere function, and telomere maintenance mechanisms. Thus, a more modern definition, which encapsulates the broad array of mechanisms by which epigenetic processes can occur, is: "the structural adaptation of chromosomal regions so as to register, signal, or perpetuate altered activity states."[1] The sum total profile of epigenetic modifications in a cell (often assessed at the tissue level) is termed the "epigenome."

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The most widely studied epigenetic mechanism is DNA methylation, which involves a covalent addition of a methyl group to a cytosine, usually in the context of cytosine-phospho-guanine dinucleotides (CpG; common abbreviations provided in Table 1).[2,3] Other epigenetic mechanisms include a suite of chromatin modifications, such as the alteration of histones, around which DNA is packaged in nucleosomes (eg, through addition of a methyl or acetyl group), replacement of canonical histones with histone variants, and chromatin modification mediated by interaction with noncoding RNAs.[4] Epigenetic mechanisms regulate all gene expression by determining the accessibility of DNA to drivers of gene activation, such as transcription factors.[2,3] As they are not part of the genome, epigenetic marks are plastic and responsive to the environment, and, in contrast to genetic mechanisms, are also cell type— and developmental stage—specific.[2,3]

The early life epigenome and postzygotic differentiation

Epigenetic mechanisms are essential for cellular differentiation, and therefore development.[5] Recent years have garnered a large interest in the epigenetic landscape of the developing pregnancy, and how epigenetic change, mediated by environmental influence, modifies the developmental trajectory and subsequent phenotype.

Prior to implantation, the epigenome is highly dynamic. Extensive epigenetic reprogramming occurs twice during mammalian development. The first genome-scale demethylation event occurs during the generation of female and male germ cells, where an erasure of epigenetic marks (including imprinted genes) occurs followed by the establishment of gamete-specific epigenetic marks.[6,7] Recent evidence from mice indicates that

epigenetic marks are not completely erased during gametogenesis.[8-12] This finding suggests a potential for transgenerational epigenetic inheritance via the gametes, although direct evidence for this phenomena remains lacking. The second event of epigenetic reprogramming occurs early postfertilization. The fertilized ovum undergoes a series of cellular divisions prior to implantation, eventually reaching the blastocyst stage.[7] DNA methylation in the zygote progressively decreases; the average methylation at fertilization is 41%, and this declines by 9% at the 2-cell stage.[13] By the blastocyst stage, the genome is almost completely hypomethylated; however, imprinted epigenetic marks remain intact during this stage.

Totipotent blastocyst cells soon proliferate and differentiate into 2 lineages, the pluripotent inner cell mass (ICM), which will become the developing fetus, and trophectoderm, from which extraembryonic tissues arise.[14,15] This differentiation involves changes in gene expression mediated by distinct epigenetic alterations, as the first de novo DNA methylation, and other epigenetic events, occur.[16,17] Evidence suggests that the reestablishment of epigenetic marks is more limited in the trophectoderm, reflected in the hypomethylation of extraembryonic tissue (ie, placenta) compared to somatic cells.[18]

The developing epigenome is sensitive to environmental influence

In the developing embryo, subtle changes in the microenvironment during specific developmental stages drive epigenetic change associated with cellular differentiation.[11] However, this inherent sensitivity to external influence, that is a hallmark of early development, also potentially renders epigenetic marks susceptible to external environmental influence.

The Developmental Origins of Health and Disease (DOHaD) hypothesis, that arose from Barker's[19] earlier fetal programming hypothesis, posits that early life exposures may alter the risk of disease of progeny in later life.[11] Barker[19] originally proposed that adverse environmental exposures in early development induce changes that, while adaptive in the short term, are potentially detrimental for long-term health. The stipulation for adaptive change in the original hypothesis, while having some merit, is limited in scope. Thus, a broader definition of the DOHaD hypothesis encompasses both maladaptive and adaptive developmental changes in response to environmental exposures with potential to influence physiology and impact later health.[19]

Unsurprisingly, epigenetic mechanisms are widely thought to mediate DOHaD-related phenomena. Several maternal exposures have now been shown to induce epigenetic change in the offspring, often in association with altered phenotype.[11,20,21] The most robust evidence is related to maternal smoking, linked to specific epigenetic changes in blood of offspring at birth, some of which is stable postnatally.[22,23] In particular, aberrant aryl-hydrocarbon receptor repressor methylation has been found in response to maternal smoking in several independent studies.[22-27] One genome-wide analysis of 790 mother-offspring dyads found a dose-dependent response of aryl-hydrocarbon receptor repressor methylation in newborn cord

TABLE 1
Glossary/abbreviations

Term	Explanation
canonical histones	core histones, as opposed to histone variants with small amounts of amino acid changes that exert regulatory functions
ccfDNA	circulating cell-free DNA
CpG	cytosine-phospho-guanine dinucleotides
CYP	cytochrome P450
DMR	differentially methylated region
DNMT	DNA methyltransferase
DOHaD	Developmental Origins of Health and Disease
EOPE	early onset preeclampsia
EVT	extravillous trophoblasts
GDM	gestational diabetes mellitus
genomic imprinting	expression of certain genes in parent-of-origin-specific manner
ICM	inner cell mass
IGF	insulin-like growth factor
IUGR	intrauterine growth restriction
LEP	leptin
LINE	long interspersed nuclear elements
LOI	loss of imprinting
LOPE	late onset preeclampsia
PE	preeclampsia
retrotransposable elements	mobile DNA sequences that can replicate themselves within genome
TE	transposable elements; DNA sequences that jump from one location to another
transcription factors	proteins involved in RNA transcription
TSG	tumor suppressor gene
VEGF	vascular endothelial growth factor

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