Published in final edited form as:

Am J Reprod Immunol. 2009 August ; 62(2): 78. doi:10.1111/j.1600-0897.2009.00716.x.

Epigenetics in the Placenta

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Abstract

Epigenetics is focused on understanding the control of gene expression beyond what is encoded in the sequence of DNA. Central to growing interest in the field is the hope that more can be learned about the epigenetic regulatory mechanisms underlying processes of human development and disease. Researchers have begun to examine epigenetic alterations – such as changes in promoter DNA methylation, genomic imprinting, and expression of miRNA – to learn more about epigenetic regulation in the placenta, an organ whose proper development and function are crucial to the health growth and survival of the developing fetus. A number of studies are now making important links between alterations to appropriate epigenetic regulation in the placenta and diseases of gestation and early life. In addition, these studies are adding important insight into our understanding of trophoblast biology and differentiation as well as placental immunology. 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. Thus, epigenetic alterations may aid in disease diagnosis and prognosis as well as in targeting new treatment and prevention strategies.

Keywords

DNA methylation; environmental exposure; miRNA; imprinting

Our understanding of the interplay between genes and the environment is being greatly enhanced in the post-genome era. There are only a few settings where the importance of this gene-environment interface is more profound than during intrauterine development, where the “critical windows” are narrower and where disruption or modification can influence fetal development as well as lead to programming of health throughout the life course. This phenomenon, now known as “fetal programming”, is a model of gene environment interaction and can inform the mechanistic basis of the synergistic effect(s) of the environment and the molecular character of development^{1, 2}.

Research in fetal programming and many other disciplines is now focusing on the paradigm that gene regulation occurs beyond the DNA sequence. Most of the acquired adverse effects related to intrauterine environment cannot be due to genetic alterations. This critical role of *epigenetic* regulation, the mitotically and meiotically heritable control of gene expression not related to DNA sequence, during development is becoming increasingly appreciated. Thus, an understanding of changes to the cellular epigenome is at the interface of the interaction between genes and environment, and can provide a mechanistic basis for the synergistic effects. Research in model systems and now expanding to human studies has suggested that the causes and consequences of a variety of pathologies are related to environmental influence on

epigenetic regulation. Examination of the specific molecular character of these epigenetic alterations in perinatal development has been less comprehensive.

Throughout the in utero development of the fetus, the placenta is of utmost importance to ensure proper growth and development. Called by some a hallmark of mammalian development³, the placenta functions to provide the fetus with nutrients, allows for waste to be transferred and ultimately excreted by the mother, and protects the fetus from what would otherwise be a type of immune detection which would result in an attack of the placenta by the maternal immune system. In addition, the placenta has a degree of metabolic and endocrine activity, is involved in secreting hormones responsible for maintaining and regulating various stages of pregnancy, and performs biochemical reactions to protect whenever possible the fetus from exposure to toxicants or other harmful chemicals⁴. All of these functions of the placenta as well as placental gene expression thus respond to and are marked by environmental insults^{4, 5}, and in many ways, the placenta can serve as a record of in utero exposure and pathology. Various compounds and drugs, including but not limited to alcohol⁶, nicotine⁷, cocaine^{8,9}, lead¹⁰, and phthalates¹¹, have been shown to cross the placenta and alter placental gene expression; some even accumulate in the placental tissue. Investigations are underway to determine changes in the genetics and epigenetics of the placenta which are characteristic of such exposures and pathological responses resulting from these exposures.

Epigenetics

Epigenetics is broadly defined as the field of research which studies changes in gene expression that are not caused by changes in the sequence of DNA¹², and the field has seen relatively rapid growth over the past few decades, accelerated by advancements in molecular biology, biotechnology, and genomics. The emergence of a new field – namely, “environmental epigenetics”¹³ – combines the traditional ways of studying epigenetics with the understanding that environmental exposures affect such epigenetic mechanisms as well. As the field of epigenetics continues to grow and be defined, there remains a central focus on examination of 4 main modes of epigenetic regulation: DNA methylation, imprinting, histone modification, and small RNA-mediated control, specifically miRNAs.

DNA Methylation

DNA methylation has become the most heavily studied mode of epigenetic regulation¹². In brief, 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”). A general rule (one that is usually, but not always, true) is that when a given stretch of cytosines in a CpG island or islands located in the promoter region of a gene is methylated, that gene will be effectively silenced by methylation; such a CpG island would be termed “hypermethylated”. Conversely, when a given stretch of cytosines in a CpG island or islands located in the promoter region of a gene is not methylated, that gene will not be silenced by methylation; the CpG island in this case would be said to be “hypomethylated”. It should also be noted that it is not the methylation of DNA itself which contributes to transcriptional repression but rather the binding of various elements (proteins that act as transcriptional repressors, proteins that block the movement of RNA polymerase, etc.) to methylated stretches of DNA that most greatly contribute to the transcriptional repression characteristic of genes with methylated CpG islands in their promoters. Researchers continue to work on attempting to decipher a type of chromatin code – one that may give scientists clues as to what degree of promoter methylation as well as interaction with histone post-translation modifications may be necessary to silence a particular gene.

Throughout the development of the embryo, important resetting of methylation patterns of germline and somatic lineages occur. Methylation throughout the genome of the zygote is almost completely removed during the cleavage phase of development; in between the implantation and gastrulation phases of development, *de novo* methylation reestablishes the developing organism's methylation patterns which, under normal conditions, are maintained throughout the rest of the organism's life^{3, 14-16}. This patterning is not limited to the embryo, but also occurs in a specific fashion in the extraembryonic lineages, although the overall levels of methylation in extraembryonic cell lineages are significantly lower than that in the somatic lineage³. Crucial for the health and survival of the organism is the need for the appropriate removal and resetting of methylation patterns during development thereby making this period a critical window during which the environment can have profound effects on the epigenetic pattern of the offspring.

Advances in technology have given researchers tools to measure changes in DNA methylation marks and patterns. Initial studies relied on the use of methylation-sensitive restriction enzymes and Southern blotting with site specific probes to characterize DNA methylation in specific genomic regions, but such techniques allowed only for examinations of methylation at specific restriction enzyme sites and required large quantities of DNA for study. Chemical modification of the DNA by sodium bisulfite, which leads to the deamination of unmethylated cytosines to uracil, but maintains methylated cytosines as cytosine allowed for the sequencing of stretches of DNA to determine cytosine methylation in a genomic region¹⁷. Relying on sodium bisulfite conversion, methylation-specific PCR (MSP) uses oligonucleotide primers which will bind and amplify sequences of sodium bisulfite modified template DNA in a methylation-specific fashion, enabling researchers to determine changes in single site DNA methylation¹⁸. Quantitative real-time PCR as well as short read sequencing such as pyrosequencing now allow for relative quantification of the methylation status at multiple CpG sites within a region, reflecting the prevalence of methylated alleles within the template DNA¹⁹⁻²¹. Sodium bisulfite conversion also lends itself to high-throughput array based approaches similar to those used for high-throughput genotyping such as Illumina Inc.'s Infinium Methylation²⁷ assay which can simultaneously determine the cytosine methylation status at >27,000 CpG sites in human samples. Immunoprecipitation of methylated DNA followed by detection of the methylated fraction through array based approaches has become common particularly in model systems²². Next generation sequencing technologies based either on sodium bisulfite modification or on the immunoprecipitation methods are the next frontier in DNA methylation detection technologies which can be applied to both human and model system samples²³, and will certainly add to our understanding of DNA methylation in the placenta.

Imprinting

Epigenetic regulation is central to the phenomenon of genomic imprinting, 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 methylation²⁴. One type of DMR is one that is differentially methylated in all tissues throughout development and is commonly called an imprinting control region (ICR) because such ICRs are hypothesized to be key regulators of imprinting in their particular chromosomal domains²⁵. The other type of DMR is one that has differential patterns of tissue-specific methylation during stages of somatic development²⁵.

Both non-coding RNAs and changes in DNA methylation at sites in DMRs are responsible for the regulation of the imprint. Although DNA methylation is involved, it does not function in a manner similar to that seen in promoter regions, but instead functions to alter the binding of specific transcription factor and/or enhancer elements which control the allele specific

expression of the region^{26, 27}. The marks of imprinting are erased in germline cells, and re-established dependent on the sex of the individual (i.e. in sperm, paternal imprints become established and in oocytes, maternal imprints)²⁸.

Imprinting has been theorized to be one of the mechanisms involved in the so-called “parent conflict” theory²⁹. The “parent conflict 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 another²⁹. 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 siblings³⁰.

Imprinted genes are thought to function in the control of embryonic development, including placental development³¹, as well as in functions later in life such as behaviors and metabolism³². Alterations to normal imprinting patterns lead to well characterized syndromes, including Prader-Willi and Angelman Syndromes related to inappropriate imprinting at chromosome 15q11. Developmental environment has also been suggested to affect the appropriate establishment of imprinted genes, particularly at chromosome 11p15 leading to Beckwith-Wiedemann Syndrome, which has been linked to the use of assisted reproductive technologies³³⁻³⁵. Although methylation patterns are critical in the regulation of genomic imprinting, their utility to examine imprinted genes is limited by the ability to determine allelic specific methylation patterns. Most often, imprinting status is determined using allele specific PCR reactions to examine allele specific expression³⁶, although new methods based on genome-wide SNP arrays are allowing for the examination of allelic-specific expression and alterations to normal imprinting status on the genomic scale^{37, 38}, and may be useful for the examination of imprinting patterns in the placenta and their association with normal fetal growth and development.

Histone modification

Modifications of the chromatin environment play key roles in epigenetic regulation of gene expression as well. One of these epigenetic regulatory mechanisms involves the acetylation, methylation, phosphorylation, and ubiquitinylation of histones, leading to regulation of gene expression^{39, 40}. The combined effects of the modification of the amino-terminal tails of core histones by acetylation, phosphorylation, and methylation play a major role in determining gene activity⁴¹⁻⁴³. A number of classes of histone methyltransferases – key enzymes involved in the transfer of methyl groups to histones – have been discovered⁴², including the H3-K4 methyltransferase 44, 45 and five H3-K9 methyltransferases 46-49. Additionally, researchers have identified a number of transcription co-activators that have characteristic histone acetyltransferase (HAT) activity and histone deacetylases (HDACs), both of which play important roles in histone modification⁵⁰.

Histone modifications can be established in particular reactions or in a sequential order^{41, 43}; recent studies have indicated that the sequential order of modifications may be gene specific^{51, 52}. Other research has suggested that site-specific combinations of covalent histone modifications may comprise a type of histone code that can not only affect the structure of chromatin but can also affect targeting of transcriptional complexes⁵³⁻⁵⁵. Such histone modifications can lead to gene activation or gene silencing, depending on the effects on transcriptional complexes. Alterations to patterns of histone modification can have a number of negative consequences, such as developmental dysregulation, X-chromosome inactivation, or might lead a number of diseases⁵⁰. Research is continuing to better define how the patterns

of histone modifications are utilized by the cell to control gene expression, as well as how these marks are involved in regulating additional epigenetic processes ⁵⁶.

Small regulatory RNAs and microRNAs (miRNA)

In the early 1990s, researchers first published observations characterizing two small regulatory RNAs, known as *lin-4* and *let-7*, which were shown to control the timing of larval development in *C. elegans* ^{57, 58}. These RNAs, initially termed “*lin-4* and *let-7* RNAs”, were initially suggested to represent a class of endogenous RNAs found in worms, flies, and mammals and since have been renamed “microRNAs (miRNAs)” ⁵⁹⁻⁶¹. Subsequent work suggested that these small regulatory RNAs could be found in plants, mammals, green algae, and viruses ⁶². Other classes of small RNAs have been found in plants, animals and fungi; small interfering RNAs (siRNAs) ^{63, 64} and Piwi-interacting RNAs (piRNAs) ⁶⁵ are two examples. miRNAs are different from these other classes of small RNAs in that they are formed from transcripts that have been shown to fold back on themselves, generating characteristic hairpin structures ⁶⁶; other small RNA classes are formed from longer hairpins (siRNAs) or from precursor forms lacking a double-stranded nature (piRNAs) ⁶⁶. Generally, as these small regulatory RNA molecules can alter gene and protein expression without altering the underlying genetic code, they too are considered critical mechanisms in epigenetic regulation.

miRNA are transcribed by RNA Polymerase II as part of transcripts called primary miRNAs (pri-miRNAs) and include 5' caps and 3' poly(A) tails ⁶⁷⁻⁶⁹. The miRNA portion of the pri-miRNA then forms a hairpin ⁶⁷. The pri-miRNA is then digested by the dsRNA-RNA-specific ribonuclease Drosha, and the hairpin that is ultimately released is called precursor miRNA (pre-miRNA) ⁷⁰. pre-miRNA has been characterized to be 70-75 nucleotides of RNA in length with 1-4 nucleotide 3' overhangs, 25-30 base pair stems, and small loops ^{70, 71}. Data have also suggested that Drosha processes either the 5' or 3' terminus of the mature miRNA, depending on which strand of the pre-miRNA associates with the RNA-induced silencing complex (RISC) ^{70, 71}. The pre-miRNA is then exported from the nucleus to the cytoplasm by a complex containing Exportin-5 (Exp5) ^{71, 72}. After arrival in the cytoplasm, the pre-miRNA is cleaved by Dicer, an RNase III superfamily member ^{70, 71}. After cleavage by Dicer, the resulting double-stranded RNA has short 3' overhangs at either end ⁷². It should be noted that only one of the two strands in the post-Dicer-processed dsRNA is the true mature miRNA; some mature miRNAs are formed from the leading strand of the miRNA strand while others are formed from the lagging strand, in a mature miRNA-specific fashion ⁷². In order to effectively control the translation of target mRNAs, the dsRNA that Dicer has processed must be separated into two strands, and the single-stranded mature miRNA has to associate with the RISC in order to be trafficked to its mRNA target ⁷³. Researchers have shown that determination of the active strand in the ds-RNA has a direct relationship with the stability of the ends of the dsRNA ^{74, 75}; in brief, their work revealed that the strand with less stable base pairing of the 2-4 nucleotides at the 5' end of the duplex associates with RISC and ultimately takes on the role as the active miRNA strand ⁷⁴.

Data have suggested that miRNA regulate gene expression by base-pairing to a target mRNA transcript; the exact mechanism for this post-transcriptional gene regulation varies depending on a number of factors, the most noteworthy of which seems to be the degree of complementarity of the miRNA to its target mRNA sequence ⁵⁹. The active strand of the mature miRNA associates more specifically with the Argonaute protein of the RISC and upon trafficking to the target mRNA, participates in post-transcriptional repression ^{73, 76}. As a general rule, a miRNA with perfect complementarity to its target mRNA will cause the degradation of the mRNA transcript through Argonaute-catalyzed mRNA cleavage ^{73, 77, 78} while miRNA with imperfect complementarity to a target mRNA will cause translational repression by blocking or altering the normal function of machinery that would otherwise aid

in translation of mRNA into protein⁵⁹. Mechanisms by which translational repression is carried out include inhibition of translation initiation and poly(A) shortening⁷⁹. Some groups have also shown data which suggest that miRNA can use a combination of both mRNA degradation and disruption of translation to carry out post-transcriptional repression⁸⁰.

miRNA have been shown to carry out important roles in a number of responses to stress and disease. Work by van Rooij and colleagues demonstrated that a cardiac-specific miRNA, miR-208, was crucial for hypertrophy of cardiomyocytes and fibrosis in response to stress and hypothyroidism⁸¹. Findings such as this have led several to hypothesize that there may be important miRNA-based therapies for heart disease that have yet to be developed⁸². Several groups have suggested roles for miRNA in preventing or even contributing to the development and progression of cancer⁸³. miR-21, shown to be upregulated in human brain tumor glioblastoma, has also been shown to have anti-apoptotic properties in human glioblastoma cells⁸⁴. Several other miRNAs have been described as having oncogenic or even tumor-suppressive characteristics, and many have been selected for use in developing cancer-specific therapies⁸³.

There exist a number of technologies and methods that have given researchers the power to measure even the smallest of changes in miRNA expression. Reverse transcription quantitative real-time PCR has been used to determine changes in the expression of particular miRNA associated with particular exposures or diseases. High-throughput assays, including miRNA microarrays, have been used to interrogate the expression of thousands of reported and predicted miRNA sequences in tissues that have a particular exposure or disease. Downstream effects of miRNA on post-transcriptional gene regulation remain more challenging and require use of bioinformatics approaches to first predict mRNA targets of specific miRNA and then confirm the effects of over- or underexpression of miRNA on that particular target. Due to the miRNAs' ultimate role in controlling protein translation, true confirmation of targets requires examination of the proteins of interest using specific antibodies, or through in-vitro approaches coupling targeted miRNA binding regions to reporter constructs. The understanding of miRNAs' role in the human placenta is in its infancy but due to the known critical role of miRNA in human development, it is certainly an attractive and exciting field of study.

DNA promoter methylation in placental genes

Researchers focusing on the placentas of non-humans have discovered that hypermethylation in the placentas of cloned cats may be associated with decreased cloning success rates⁸⁵ and that a particular cytochrome P450 gene is controlled, in part, by varying methylation status in the placentas of sheep and cattle⁸⁶. In humans as early as the mid-1980s, researchers demonstrated the effects of site-specific DNA methylation on the binding ability of DNA-binding protein in human placenta⁸⁷, contributing greatly to understanding the effects of DNA methylation patterns on the recruitment of DNA binding elements and subsequent effects on transcription⁸⁷.

Some have further analyzed mechanisms of human placentation in the context of its similarities with human cancer progression and tumorigenesis. More specifically, data have shown that the epigenetics related to normal human placental invasion and function have striking similarities to particular patterns of tumor-associated methylation involved in the coordinated set of epigenetic silencing events at play in tumorigenesis and progression⁸⁸.

Still others 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 trophoblast 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 ⁸⁹. 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 ⁹⁰.

Others have investigated placenta-specific methylation patterns which maximize the bioavailability of essential vitamins, specifically vitamin D, at the fetomaternal interface ⁹¹. Park and colleagues have even demonstrated the association of folate and homocysteine levels and DNA methylation levels in the human placenta ⁹²; more specifically, their data suggest that maternal levels of folate and homocysteine, as well as a particular polymorphism in MTHFR 677, influence DNA methylation patterns in the placenta during pregnancy ⁹².

Taken collectively, these efforts have provided the scientific and clinical community with a better understanding of the mechanisms and pathways underlying DNA methylation's involvement in epigenetic regulation of key placental processes.

Imprinting in the placenta

It is thought that 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 pathologies⁹³. At the same time, less well characterized are the role that imprinting alterations may play in more common, placental-related pathologies including intrauterine growth restriction and preeclampsia. Some groups have focused on characterizing patterns of imprinting in the human placenta in hopes of finding new biomarkers that could be affected by prenatal conditions. More specifically, Lambertini and colleagues have utilized techniques to measure loss of imprinting (LOI) in genes in human placentas and have concluded that not only is LOI common in human placentas but may also serve as a key biomarker for epigenetics affected by prenatal conditions or environment ⁹⁴. Guo and colleagues have investigated gene expression and methylation patterns of imprinted regions in small for gestational age (SGA) placentas and have shown that loss of imprinting at H19 due to methylation alterations and subsequent effects on gene expression may be some causes of poor growth of the human fetus ⁵.

Haycock and colleagues used a mouse model to investigate the effects of imprinting control disruption at the H19/IGF2 domain as a mechanism of aberrant growth in the event of ethanol exposure during fetal development ²⁴. Their results suggested that paternal alleles in placentas treated with ethanol exhibited a significantly lower degree of methylation than those in placentas treated with saline control. Analysis of data showing a relationship between placental weight and ethanol treatment further revealed a possible relationship between DNA methylation at the CCCTC-binding factor site on the paternal allele in placentas ²⁴.

McMinn and colleagues conducted a rather thorough investigation of associations of intrauterine growth restriction (IUGR) with altered patterns of expression of imprinted genes ⁹⁵. They performed a genome-wide survey which suggested what they termed an “unbalanced” expression of imprinted genes in IUGR placentas compared to the imprinted gene expression in non-IUGR placentas. In addition, they also demonstrated a degree of differential expression of non-imprinted genes in IUGR vs. non-IUGR placentas ⁹⁵. Their research suggested that differential expression of a panel of imprinted genes may be a possible biomarker for IUGR.

Collectively, these results suggest that environmental insults and factors associated with diseases such as IUGR may impact imprinting control mechanisms in the placenta. More work needs to be done to gain a better understanding of more specific mechanistic effects of such

exposures on mechanisms of imprinting in the placenta and downstream consequences in fetal growth and development.

Histone modification in the placenta

Examinations of histone post-translational modification are focused currently on both trophoblast biology and differentiation, as well as in placental immunology. Kimura and colleagues investigated the acetylation and methylation patterns of core histones within and flanking the human growth hormone (hGH) multigene cluster in human placental chromatin⁴⁰. Their data further distinguished the differences between placental and pituitary mechanisms of transcriptional control at the hGH cluster and suggested that the selective nature of placental gene activation seems to point toward unique roles for histone acetyltransferase and histone methyltransferase coactivator complexes in the regulation of placental gene expression⁴⁰.

Morris and colleagues utilized chromatin immunoprecipitation (ChIP) assays to further investigate transcription factor assembly and histone modifications that occur during gamma interferon (IFN-gamma) induction of the master regulator of major histocompatibility complex class II (MHC class II) transcription, known as CIITA⁹⁶. Treatment of most cells with IFN-gamma causes the induction of MHC class II genes; however, trophoblast cells will not upregulate MHC class II following exposure to IFN-gamma⁹⁷⁻⁹⁸. Such inability to upregulate MHC class II genes when exposed to IFN-gamma has been suggested to be one possible mechanism relating to maternal-fetal tolerance, in which the mother's immune system does not react to placental tissues that are expressing paternally-derived (and thus recognized as foreign) genes⁹⁶.

As described by Morris and colleagues, the major IFN-gamma responsive promoter for the expression of CIITA, Promoter IV (PIV), needs both STAT1 and IFN regulatory factor 1 (IRF-) for induction by IFN-gamma. The investigators first noted the binding of STAT1 to PIV and also noted the association of a medium degree of histone H3 and H4 acetylation⁹⁶. However, CIITA expression was not detected until IRF-1 protein was synthesized and properly bound to its necessary site. When using fetal trophoblast-like cell lines that are resistant to CIITA induction by IFN-gamma, the authors observed that the cells could not properly assemble factors which would otherwise enable them to be inducible by IFN-gamma; moreover, these cells were also unable to modify their chromatin, a characteristic which suggested that the promoter region may have been blocked. Subsequent experiments showed that PIV exhibited a strong degree of hypermethylation. Taken collectively, Morris and colleagues' data furthered the understanding of the activation of the CIITA gene in response to IFN-gamma and suggest that assembly of regulatory factors, modification of chromatin, and expression of genes are linked and may progress in a distinct series of steps⁹⁶.

Other groups, such as Chuang and colleagues, 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 fusion⁹⁹. Chuang and coworkers 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 HDAC⁹⁹.

Collectively, research into the effects of histone modification on placental gene expression continues to unveil a better understanding of this important epigenetic regulatory mechanism. Such knowledge has enabled researchers to gain a better understanding of important phenomena such as maternal-fetal tolerance and placental differentiation and growth.

Placental miRNA

Several groups have published data demonstrating that miRNA expression is tissue-specific and that several miRNAs are expressed in the human placenta^{80, 100}. Since such discoveries, 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.

Pineles and colleagues measured miRNA levels in placentas to investigate whether placental miRNA expression patterns are associated with preeclampsia or small for gestational age diagnoses¹⁰¹. This work revealed differential expression of miR-210 and miR-182 in preeclampsia versus control patients and showed key associations in miRNA expression patterns and preeclampsia, furthering efforts to find a miRNA biomarker of preeclampsia pathology¹⁰¹. Zhu and coworkers found that thirty four miRNAs were differentially expressed in preeclamptic placentas compared to normal placentas further suggesting a role for miRNA in the pathogenesis of preeclampsia¹⁰². Such biomarkers would prove especially useful in giving clinicians and pathologists sensitive molecular tools to better diagnose preeclampsia, a disorder that has great impacts on both pregnant women and their newborns.

It has long been speculated that 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. To investigate this further, Donker and coworkers analyzed the relationships between expression of Argonaute 2, an important RNAi enzyme, and other miRNA in trophoblasts and in environments with varying oxygen level¹⁰³. Donker and colleagues' 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 oxygen¹⁰³. 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 groups have observed and detected placental miRNA in maternal plasma and have conducted comparative studies on circulating miRNA and other circulating nucleic acids (CAN) in the maternal sera of pregnant and non-pregnant women^{104, 105}. Chim and colleagues showed that placental miRNAs (miR-141, miR-149, miR-299-5p, and miR-135b) were highly expressed in maternal plasma in pregnant mothers and suggested that such expression patterns could serve as important biomarkers for monitoring pregnancy¹⁰⁴. Gilad and coworkers demonstrated that placental miRNA levels in sera from pregnant women were higher than those in sera from non-pregnant women; moreover, levels of miRNA in sera of pregnant women correlated with pregnancy stage¹⁰⁵. Thus, placental miRNA levels detectable in maternal serum may serve as important clinical biomarkers of pregnancy, pregnancy stage, and other pregnancy-related outcomes.

Future Directions in Placental Epigenetics

Data have suggested that environmental exposures can alter miRNA expression in in-vitro systems¹⁰⁶ and various tissues¹³ and that altered levels of miRNA expression can be associated with particular diseases or risk factor for disease¹⁰⁷. As reviewed above, work has also been done to investigate biomarkers of diseases, such as cancer, growth retardation, and other records of in utero environment in placental patterns of DNA methylation, imprinting, histone modification, and miRNA expression. Studies aimed at further revealing the importance of miRNA in responding to environmental exposure and to disease may prove very useful in better understanding how such exposures and diseases affect the body. Current and past research into diseases of pregnancy – such as finding miRNA associated with preeclampsia^{101, 102} or gene-specific placental methylation patterns associated with GTD⁸⁹ – might be expanded to include

work to better understand associations between epigenetic factors in the placenta and diseases such as choriocarcinoma, childhood cancers, and other diseases of childhood and adolescence, as well as alterations to normal placental immunology and function.

Using the placenta as a reference tissue allows researchers to utilize an important residual tissue whose respective miRNA expression and DNA methylation patterns may prove to be powerful biomarkers possessing predictive capability for a number of diseases or disease progression. Modern advances in bioinformatics – such as pathway analysis tools and target gene prediction software – as well as advances in technology, such as microarray technology, have given researchers and clinicians tools to better detect patterns in DNA methylation, imprinting, histone modification and miRNA expression that are associated with particular exposures and diseases. Aberrant patterns of miRNA expression or DNA methylation may ultimately serve as biomarkers for exposure, disease burden, or even as “early indicator” diagnostics of increased risk for developing future disease or disorders.

Conclusion

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. Such biomarkers may prove essential for developing new diagnostics for early diagnosis of risk factor and levels of exposure. Additionally, these aberrant patterns of miRNA expression, imprinting, DNA methylation, or histone modification may identify previously unknown pathways targeted for alteration, which, in turn, may serve as targets for novel drug treatment or prevention strategies. These epigenetic biomarkers can be brought from the benchtop to the bedside and will be useful in helping clinicians better diagnose and prevent the onset of disease.

Acknowledgments

This work is supported by grants from the NIH-NCRR (P20RR018728), the NIEHS Superfund Basic Research Program (P42ES013660), the NIEHS Training Program in Environmental Pathology (T32ES007272, MAM) and the Flight Attendants Medical Research Institute.

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