Genomic imprinting in the human placenta

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With the launch of the National Institute of Child Health and Human Development/National Institutes of Health Human Placenta Project, the anticipation is that this often-overlooked organ will be the subject of much intense research. Compared with somatic tissues, the cells of the placenta have a unique epigenetic profile that dictates its transcription patterns, which when disturbed may be associated with adverse pregnancy outcomes. One major class of genes that is dependent on strict epigenetic regulation in the placenta is subject to genomic imprinting, the parent-of-origin-dependent monoallelic gene expression. This review discusses the differences in allelic expression and epigenetic profiles of imprinted genes that are identified between different species, which reflect the continuous evolutionary adaption of this form of epigenetic regulation. These observations divulge that placenta-specific imprinted gene that is reliant on repressive histone signatures in mice are unlikely to be imprinted in humans, whereas intense methylation profiling in humans has uncovered numerous maternally methylated regions that are restricted to the placenta that are not conserved in mice. Imprinting has been proposed to be a mechanism that regulates parental resource allocation and ultimately can influence fetal growth, with the placenta being the key in this process. Furthermore, I discuss the developmental dynamics of both classic and transient placenta-specific imprinting and examine the evidence for an involvement of these genes in intrauterine growth restriction and placenta-associated complications. Finally, I focus on examples of genes that are regulated aberrantly in complicated pregnancies, emphasizing their application as pregnancy-related disease biomarkers to aid the diagnosis of at-risk pregnancies early in gestation.

Key words: assisted reproductive technology, chromosome cluster 19, differentially methylated regions, DNA methylation, epigenetics, fetal growth, imprinting, intrauterine growth restriction, microRNA, MiRNA, placenta specific expressed genes, preeclampsia

The placenta is a transitory organ that ensures appropriate fetal growth and survival in utero. This unique organ is essential for the production of pregnancy-related hormones, the transport of nutrients and waste between the maternal blood and the developing fetus, and the assurance of protection from the maternal immune system. The placenta initiates from the trophectoderm of the blastocyst and results in the fetal chorion, which along with the maternal decidua forms the placenta bed. Placenta villi are composed of 3 main layers with different cell types that include syncytiotrophoblast/cytotrophoblast cells that cover the surface of the villous tree, mesenchymal cells that are located within the villous stroma, and the fetal vascular cells that include smooth muscle and endothelial cells. The gene expression patters of the fetal and maternal components of the developing placenta differ from each other, exhibiting temporal changes during development.[1,2] Normal expression within the placenta is essential for normal pregnancy, with aberrations that are associated with pathologic conditions such as intrauterine growth restriction (IUGR) and preeclampsia.

The mechanisms that lead to IUGR are not understood completely, but abnormalities of the maternal-fetal circulation have been implicated because of the observed abnormalities in Doppler velocimetry of the uterine and umbilical arteries, which are consistent with increased impedance to flow in the maternal spiral arteries and the placental circulation, respectively.[3-5] Pregnancies that are complicated by IUGR often require early elective delivery if the fetus is suspected to be in distress, which results in additional complications that are associated with prematurity.[6] In addition, IUGR neonates not only have immediate medical problems but also are at increased risk of hypertension, type 2-diabetes mellitus, and heart disease in adult life through the effects of fetal programming, which commonly is referred to as the "fetal origins of adult disease."[7] Despite considerable research in this area, it is unclear how these changes lead to growth and metabolic diseases later in life. One attractive hypothesis is the involvement of manipulable epigenetically regulated gene activation and repression.

Imprinting

In mammals, imprinted genes are known to regulate placental development and fetal growth and are believed to have coevolved with placentation.[8] Most mammals express their autosomal genes codominantly from the 2 parental chromosomes. However, at some loci (the total number yet to be defined), the allele inherited from one parent is suppressed through an epigenetic mechanism that results in imprinted monoallelic parent-of-origin- specific expression. This monoallelic expression is achieved by epigenetic asymmetries between parental alleles that include

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differential DNA methylation, histone modifications, antisense noncoding RNA (ncRNA)—mediated silencing, and long-range chromatin interactions (Table 1). Based on current reports it is assumed that there will be 100-200 genes that are subject to imprinted expression in mammals, with many being tissue-specific.[9]

There is no unified explanation of how imprinted genes evolved during mammalian development, but several theories have been hypothesized. Genomic imprinting occurred 210-310 million years ago after the divergence of monotremes from marsupials and placental mammals.[10] This restriction to therians reinforces the evolutionary link of imprinting with placentation, at least in mammals. Indeed, the phenotypes of androgenetic and gynogenetic mouse embryos, as well as that of complete androgenetic hydatidiform moles in humans, clearly indicates a role of imprinted genes in trophoblast differentiation and early placenta development.[11-13] As a result, these observations have been the basis of many theories for the evolution of imprinting, including its function as a protective mechanism against excessive placentation and ovarian teratomas.[14,15]

However, the most popular hypotheses are associated with parental conflict and the nutrient supply and demand hypothesis.[16,17] This theory implies that imprinting has evolved because of conflict of resource provisions by the mother to her offspring through the placenta and that paternally expressed genes are "selfish" and are selected to demand nutritional resources from the mother, whereas maternally expressed genes have to balance the nutrition provision to the current fetus with that of future fetuses of the mother (and potentially different fathers).[18] In reality, there is probably not a single unifying reason why imprinting arose in mammals, and in fact a combination of multiple theories may most appropriately explain the evolution of imprinting.

Allelic expression strategies

The identification of imprinted genes traditionally has been performed in mouse because of the ease of embryo and genetic manipulations and has used gynogenetic and androgenetic embryos or mice harboring regions of uniparental disomy, where 2 copies of an entire chromosome or chromosomal region are inherited from only 1 parent.[19] In the past, these embryos have been used in expression screenings such as subtractive hybridization, differential display, or expression array hybridization.[20,21] However, with the advent of next generation sequencing technologies, new strategies are based on quantitative whole-transcriptome sequencing (messenger RNA-sequencing) and the use of single nucleotide polymorphisms to discriminate alleles.[22-25] With the use of such an approach, imprinting would be observed as a skewed genotype call in the RNA at a position heterozygous in the corresponding DNA sample, with allelic calling based on the genotype of parental samples. This approach has been used in both mouse and humans to identify candidates that exhibit spatiotemporal imprinted expression patterns.[26] However, such approaches are fraught with problems, including the necessity of complex bioinformatics analysis and the fact that this technology relies on relatively short sequence reads that may not be compatible with isoform-specific imprinting, because splicing events and a polymorphism must be within the same sequence read.

Transcriptome sequencing has been applied successfully to the identification of novel imprinted genes in both mouse and human placenta samples. However, unlike other somatic tissues, studies in placenta are associated with an important confounding effect: the contamination of maternally derived decidua that will result in false-positive maternal expression being identified. This is relevant especially to studies in mice because decidual cells can remain after careful dissection. Recently, Okae et al[24] performed experiments using placenta samples from reciprocal mouse hybrids in which they identified hundreds of genes expressed predominantly from the maternal allele; however, the vast majority was associated with contaminating decidual cells (Table 2). These elegant studies also revealed that some placenta-specific maternally expressed genes are truly imprinted despite high expression in decidua, for example *Tfpi2*.[27] It is possible to distinguish false-positive maternal expression because of decidua contamination from true imprinting by utilizing in situ staining or backcrosses in which homozygous embryos are produced in a heterozygous mother. In this situation, detection of the allele not present in the genome of the embryo will signal the existence of maternal contamination.[28] Such approaches have revealed that several imprinted genes that previously have been reported to exhibit placenta-specific maternal expression, including *Gatm* and *Dcn*, result from maternal cell contamination.[24] These observations are endorsed by the fact that no regions of differential DNA methylation or repressive histone modifications have been identified in the vicinity of these genes.[29-31] Addition approaches have been used to overcome this problem, including the isolation of specific cell lineages with the use of immune selection. Such procedures have revealed the robust maternal expression of *GRB10* in cytokeratin 7 positive trophoblasts, despite only skewed maternal expression being observed in unselected placenta biopsy specimens.[32]

Searching for differentially methylated regions

Screening for genomic loci that harbor allelic differences in DNA methylation overcomes the problems that are associated with analyzing temporal and spatial expression because all imprinted domains to date contain imprinted differentially methylated regions (DMRs). Sophisticated screens that use techniques such as restriction landmark genomic screening, methylation-sensitive representation difference analysis, and methylated DNA immunoprecipitation have been used traditionally to identify imprinted DMRs in mice and humans.[33-36] However, the recent technologic advances in bisulphite conversion-based techniques coupled with next generation sequencing (methyl-seq or reduced-representational bisulphite sequencing) have allowed for extensive descriptions of parent-of-origin