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Imprinted and X-linked non-coding RNAs as potential regulators of human placental function

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Pregnancy outcome is inextricably linked to placental development, which is strictly controlled temporally and spatially through mechanisms that are only partially understood. However, increasing evidence suggests non-coding RNAs (ncRNAs) direct and regulate a considerable number of biological processes and therefore may constitute a previously hidden layer of regulatory information in the placenta. Many ncRNAs, including both microRNAs and long non-coding transcripts, show almost exclusive or predominant expression in the placenta compared with other somatic tissues and display altered expression patterns in placentas from complicated pregnancies. In this review, we explore the results of recent genome-scale and single gene expression studies using human placental tissue, but include studies in the mouse where human data are lacking. Our review focuses on the ncRNAs epigenetically regulated through genomic imprinting or X-chromosome inactivation and includes recent evidence surrounding the *H19* lincRNA, the imprinted C19MC cluster microRNAs, and X-linked miRNAs associated with pregnancy complications.

Introduction

The best-known function of the placenta is to mediate fetal-maternal exchange throughout pregnancy, but it also plays a major role in directing maternal adaptation to pregnancy by secreting a variety of steroid and peptide hormones that modulate maternal physiology without which pregnancy could not be sustained. The placenta is a unique organ in several respects. First, although the placenta is a shared organ between mother and fetus, it is an extra-embryonic tissue and is therefore primarily regulated by the fetal genome. Second, the placenta completely separates from mother and fetus after birth, making it the only truly transient organ. For this reason, the placenta may not be under the same lifetime epigenetic constraints as other somatic tissues. Placental development in humans begins shortly after an embryo implants into the lining of the uterus, where it begins a strikingly invasive process that remodels the uterine spiral arterioles to sequester a maternal blood supply to facilitate efficient feto-maternal exchange. This invasive process, which has many similarities with cancer metastasis,[1] appears to be strictly controlled both spatially and temporally in humans through mechanisms that are only partially understood.[2,3] However, emerging evidence, particularly from high-throughput gene expression technologies, suggests non-coding RNA molecules (ncRNAs) direct and regulate a considerable number of biological processes and cellular functions. Therefore, ncRNAs may constitute a previously hidden layer of regulatory information in the placenta. In this review, we focus on the imprinted and X-linked ncRNAs, which are typically expressed from only one allele. We explore the regulation of these ncRNAs in the context of human placental development. Examining particular influential genomic regions, a key focus of this review will be the role that ncRNA expression in the placenta plays in pregnancy complications, such as preeclampsia, that are attributed to abnormal placental development. Although this review is focused on human placental development, studies in the mouse are also discussed where human data are lacking.

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The Placenta is Key to Fetal and Maternal Health

The placenta is part of the conceptus and therefore is genetically identical to the fetus. Its development is initiated at implantation about 5–6 d after conception and follows a dynamic and constantly changing trajectory providing gaseous and nutrient exchange functions between the maternal and fetal circulations to support fetal growth.[4] Impaired placental (trophoblast) invasion has been implicated in several complications of pregnancy such as preeclampsia and intrauterine growth restriction (IUGR)[5] and pre-term labor.[6,7] For example, in preeclampsia, invasion of the spiral arterioles and the maternal decidual stroma is shallow, resulting in poor maternal blood flow to the placenta.[5,8,9] Despite huge research efforts, our understanding of the highly complex molecular regulation of both normal and abnormal placentation is still inadequate. However, ncRNAs are emerging as key regulators of development[10,11] and therefore provide new avenues of inquiry relating to placental differentiation and function. If so, the perturbed regulation of ncRNAs in the placenta may result in one or more of a continuum of pregnancy complications that compromise the health of both mother and infant.

Classification and Detection of Non-Coding RNA

There are many different classes of ncRNAs, as these molecules vary greatly with regards to sequence length and complexity, splicing isoforms, polyadenylation, regulation, and biological function. The most well-characterized class of ncRNAs are the infrastructural RNAs (rRNAs, tRNAs, snRNAs, snoRNAs), which constitute many integral cellular components and are involved in processes such as translation, transcript splicing and higher level regulatory processes, including DNA methylation.[10] Other ncRNAs are typically classed based on their sequence length, with RNAs shorter than ~200 nucleotides termed short non-coding RNAs (sncRNAs), and those greater than ~200 nucleotides are termed long non-coding RNAs (lncRNAs).[11] The sncRNAs include the microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), and the small interfering RNAs (siRNAs). These short RNAs, particularly the miRNAs, have received the most attention to date, and currently dominate the ncRNA literature. However, there has been a steady accumulation of evidence indicating that lncRNA transcripts, as a class, have a diverse repertoire of biological functions[11] and constitute a significant proportion of total cellular RNA.[12]

Although the central dogma of biology has previously allowed little scope for the regulatory capabilities of ncRNA (for a review see ref. 13), the ability to detect and measure ncRNAs has also hindered progress toward appreciating the gamut of their functional abilities. Detecting ncRNAs in any tissue has posed challenges for several reasons. First, distinguishing if a transcript has protein-coding ability can be difficult as ncRNA transcripts can originate from intronic and untranslated regions of coding transcripts, or can be alternative splice variations that abolish a transcript's coding potential.[14,15] Second, lncRNAs can be transcribed from DNA that spans intergenic and coding regions resulting in transcripts that host protein-coding DNA sequence. Third, many ncRNAs do not end with a poly-A signal,[12] which is characteristic of protein coding genes. This difference has profound implications regarding ncRNA detection as many cDNA, SAGE, microarray, and RNA-Seq methods rely on poly-A labeling, enrichment or priming. For these reasons among several others (see ref. 16), ncRNA transcripts can be difficult to discover and measure, which has subsequently hampered our ability to annotate and functionally classify ncRNAs in health and disease.

The Roles of Non-Coding RNAs in Genomic Imprinting in the Placenta

Imprinted genes are known to have significant effects on placental development and are implicated in many placental pathologies.[17–19] Imprinted genes are expressed in a parent-of-origin-dependent manner, with the imprinted alleles being epigenetically silenced.[20,21] Genomic imprinting is typically observed in clusters of ~2–12 genes, with most of these clusters having at least one lncRNA gene.[22] The epigenetic regulation of imprinting can involve DNA methylation imprints, repressive histone modifications, and complex enhancer competition scenarios involving *cis*-acting lncRNA transcripts.[22–25] Ablation of lncRNAs within imprinting clusters typically results in the loss of imprinting,[22] demonstrating that lncRNAs can act as *cis* regulators of autosomal gene expression.

Imprinting is largely, although not exclusively, observed in eutherian mammals and is thought to have arisen with viviparity and the evolutionary emergence of the placenta.[26,27] The prevailing evolutionary hypothesis of imprinting suggests that paternally-expressed genes have been selected to maximize fetal resource acquisition from the mother, while maternally-expressed genes have been selected to balance resources allocated to current and future offspring.[27] Since imprinted genes are suggested to facilitate a tug-of-war between maternal and paternal genomes, this hypothesis predicts that imprinted genes are heavily involved in fetal and placental growth and development throughout pregnancy.[21,27,28] Not surprisingly, more imprinted genes are expressed in the placenta than in any other tissue, with several being placenta specific.[29]

Although the exact mechanisms regulating imprinted regions remain unclear, the maintenance of imprints appears to differ between embryonic and extra-embryonic tissues.[29] This suggests that extra-embryonic cell lineages, many of which make up the placenta, may employ regulatory mechanisms involving ncRNAs that are not observed in embryonic cell lineages. Despite the fact that much of our understanding of placental imprinting comes from studies in mice,[29] the evidence from human research to date suggests that many human placental abnormalities and pregnancy complications are associated with altered imprinting involving ncRNAs.

The Imprinted *H19* Long Non-Coding RNA and miR-675

H19 was one of the first lncRNAs to be discovered and is considered a key regulatory molecule in placental development. *H19* lies within a large imprinted domain (>1 MB), and is predominantly expressed from the maternal chromosome. *H19* placental expression is largely monoallelic[30] and is one of the most highly expressed genes in the human placenta.[31] However, the functional roles of *H19* are only now beginning to emerge.

H19, and the adjacent and reciprocally-imprinted *IGF2* gene, make up one of the most widely studied imprinted genomic regions in humans. Both *H19* and *IGF2* share many *cis*-regulatory elements, with the prevailing regulatory model of this locus indicating a complex interaction of DNA methylation, CTCF binding and enhancer competition scenarios mainly elucidated through targeted deletion and transgenic techniques in murine models.[32]

Somewhat consistent with observations in humans, studies in mice have further demonstrated that altered imprinting of *H19* is associated with placental and fetal growth abnormalities.[32–34] For example, (epi)mutations in the *H19–IGF2* region are associated with Silver-Russell and Beckwith-Wiedemann syndromes, which manifest phenotypically in utero as severe growth-restriction and overgrowth, respectively.[35] Furthermore, altered epigenetic regulation of the *H19-IGF2* region in human placentas has

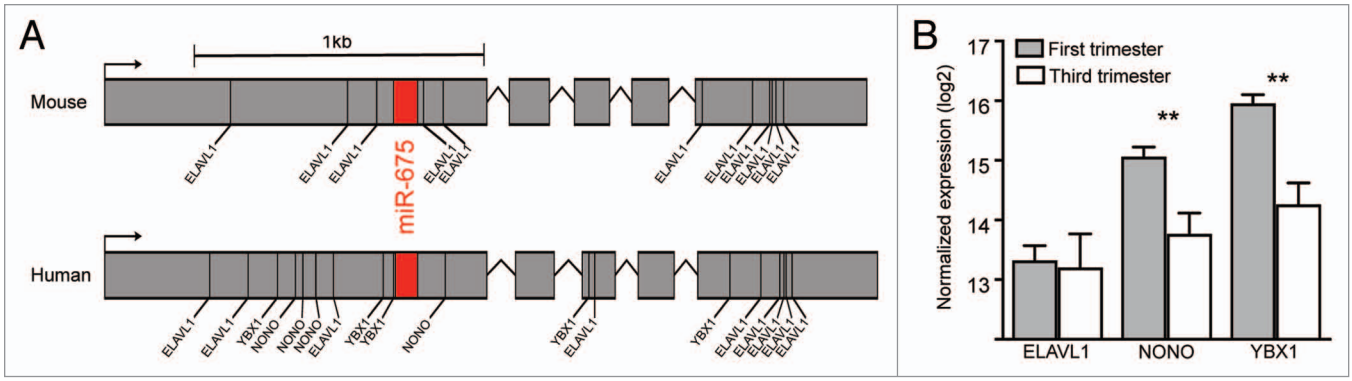

Figure 1. The *H19* lncRNA transcript and RNA binding proteins in human and mouse. (**A**) Schematic representation of human and mouse *H19* transcripts indicating locations of RNA binding protein motifs. The ELAVL1 binding motifs that surround the miR-675 locus in the mouse transcript are not present in the human transcript, however binding motifs for the NONO and YBX1 proteins are present in the human transcript. (**B**) Expression of genes that encode the RNA binding proteins *ELAVL1*, *NONO*, and *YBX1* in human first and third trimester placentas. Expression of *NONO*, and *YBX1* show a significant decrease in expression as gestation progresses, with *ELAVL1* showing no difference across gestation. Data for RNA binding protein expression differences between first and third trimester were reported in reference 44 and the figures were generated using normalized array data obtained from the NCBI Gene Expression Omnibus (accession GSE28551). The RNA binding protein recognition sequences were predicted using the RNA binding protein database.[107] Human and mouse *H19* transcript sequences obtained from UCSC reference genomes hg19 and mm10 respectively.

been associated with the pregnancy complication preeclampsia, which is attributed to abnormal placental development early in gestation.[36,37] Biallelic expression of *H19* has been observed at higher rates during the first trimester of pregnancy compared with term,[36,38,39] with the early first trimester placenta showing patterns of imprinting plasticity.[30] Together, these studies suggest *H19* plays an important regulatory role in early placental development.

Recent work suggests that *H19* is a *trans* regulator of an imprinted gene network for growth and development[40] involving miRNAs hosted within the *H19* transcript,[32,41,42] which may account for some of *H19*'s bioactivity. Most recently, Keniry et al. have described *H19* as a developmental reservoir of miR-675 in the mouse.[43] This study shows the miR-675 microRNA is processed from the first exon of *H19* in a developmental stage specific manner in the placenta. They also showed that levels of miR-675 increased with gestation acting as a placental growth suppressor.[43] Although overall *H19* expression remained unchanged throughout gestation, the RNA-binding protein Elavl1 (also known as *HuR*) appeared to bind to the *H19* transcript preventing excision of miR-675 early in gestation.[43] Elavl1 abundance decreased as gestation progressed, enabling miR-675 to be processed and to act as a placental growth suppressor.[43] Although this study has increased our knowledge of *H19* function in the placenta, it may not accurately portray the situation in humans for several reasons. First, the human and mouse *H19* transcripts show notable sequence divergence. Second, a microarray analysis by Sitras et al. found no significant difference in *ELAVL1* expression between first trimester and third trimester human placentas,[44] (**Fig. 1A**) which is contrary to the observation in mice. However, as suggested by Keniry et al., the excision of miR-675 may also be regulated by additional RNA binding proteins.[43] To examine this possibility, we performed an in silico analysis of RNA binding protein domains within the human and mouse *H19* transcripts. We note that the ELAVL1 binding sites that flank the miR-675 locus in mouse are not present in the human transcript (**Fig. 1A**). However, we observed that binding domains flanking miR-675 existed for the RNA binding proteins NONO and YBX1 in the human *H19* transcript, and these proteins show a significant decrease in expression as gestation progresses (**Fig. 1B**). These differences between mouse and human indicate further work is required to elucidate the true extent of *H19* and miR-675 regulation and functionality in the human placenta. This would require miR-675 expression across human gestation to be evaluated, followed by a careful analysis of how miR-675 excision is repressed in humans. These experiments using human derived samples will be a fundamental step toward determining why *H19* is implicated in human pregnancy complications attributed to abnormal placental development.

The Imprinted C19MC miRNA Cluster

An intriguing observation of placental-expressed miRNAs arises from the largest known miRNA cluster discovered to date; C19MC. This cluster, located at human chromosome 19 (19q13.42), features ~46 miRNA genes transcribed from a ~100 kb region. C19MC is imprinted, with only the paternally inherited chromosome being expressed[45,46] predominantly, if not exclusively, in the placenta.[47] Furthermore, C19MC is unique to the primate lineage, excluding model organism research to determine the functions of miRNAs in this cluster.[47]

Transcription of C19MC miRNAs can be activated in some cells by treatment with DNA methylation inhibitors indicating that the region is under DNA methylation-dependent epigenetic control.[45,48,49] Further evidence also suggests that the C19MC miRNAs are excised from a much larger lncRNA, which is transcribed from an RNAP II promoter within a CpG-rich region.[45,47] C19MC miRNAs are also expressed in exosomes released from primary human trophoblast cells and are detectable in the serum of pregnant women,[50] highlighting their potential as

Table 1. Imprinted or X-linked miRNA differentially expressed in preeclampsia (PE) and pre-term birth (PTB) with validated targets and potential mechanisms

Pregnancy complication	miRNA (cytoband)	Expression in complication vs. control	Experimentally validated target genes[109,110] detectable in the human placenta[31]	Potential roles and contributing mechanisms
PE	miR-20b[53,54,102] (Xq26.2)	Increased	*ARID4B, BAMBI, CDKN1A, CRIM1, ESR1, HIF1A, HIPK3, MYLIP, PPARG, STAT3, VEGFA*	Impairing placental function through suppression of genes (*CRIM1*, *HIF1A, VEGFA*) that have a role in maintaining endothelial cell function and angiogenesis.[104,111–113] Repression of genes (*CDKN1A, HIPK3, STAT3*) involved in apoptosis and trophoblast invasion.[114–116]
PE	miR-222[54,103] (Xq11.3)	Increased	*BBC3, BCL2L11, CDKN1B, CDKN1C, CORO1A, ESR1, FOS, FOXO3, ICAM1, MMP1, PPP2R2A, PTEN, SOD2, SSSCA1, STAT5A, TCEAL1, TNFSF10, TP53*	Downregulation of genes (*BBC3, BCL2, CORO1A, FOS, FOXO3, TNFSN10, TP53*) that can promote apoptosis.[117–122] Downregulation of genes (*ICAM1*) involved in endothelial cell function.[123] Downregulation of genes (*CDKN1B, CDKN1C*) that regulate cell cycle progression and trophoblast differentiation.[124]
PE	miR-223[52,53] (Xq12)	Decreased	*CHUK, Il6, IRS1, LMO2, NFIX, RHOB, STMN1*	Upregulation of a gene (*RHOB*) involved in apoptosis signaling.[125] Upregulation of a gene (*IL6*) involved with immune response and inflammation.[126]
PE	miR-519b[53,54] (19q13.42)	Increased	*CDKN1A, ELAVL1*	Alteration of apoptosis signals (*CDKN1A*).[114] Downregulation of *ELAVL1*, potentially altering miR-675 excision from the *H19* lincRNA.[43]
PE	miR-519e[52,53] (19q13.42)	Increased	*CDKN1A*	Alteration of apoptosis signals (*CDKN1A*).[114]
PE	miR-520 g[53,54] (19q13.42)	Increased	*VEGFA*	Impaired endothelial cell function and angiogenesis (*VEGFA*).[104]
PE	miR-524[53,102] (19q13.42)	Increased	-	-
PE/PTB	miR-517a[52,65] (19q13.42)	Increased	-	Regulation of apoptosis[127]
PE/PTB	miR-518b[52,53,65,128] (19q13.42)	Increased	-	
PE/PTB	miR-520h[53,128] (19q13.42)	Increased	*ABCG2, CDKN1A, ID1, ID3, SMAD6, VEGFA*	Downregulation of a gene (*ABCG2*) involved in protecting fetal exposure to xenobiotics ingested by the mother.[129] Alteration of apoptosis signals (*CDKN1A*).[114] Impaired endothelial cell function and angiogenesis (*VEGFA*)[104]
PE/PTB	miR-526b[53,65] (19q13.42)	Increased	-	-

fetal-maternal signaling molecules that may modulate maternal adaptation to pregnancy. Although the precise functional mechanisms of C19MC miRNAs remain largely unknown, the abundance of C19MC transcripts in the placenta, their imprinted regulation, and detection in the maternal circulation, suggest a significant role in placental biology.

Studies of pregnancy complications attributed to abnormal placental development, in particular those focusing on placental gene expression in preeclampsia where a transcriptome-wide method (microarrays or high-throughput RNA sequencing) is employed, have shown differential regulation in the placental expression of some miRNAs in the C19MC family (**Table 1**).[51–54]

Together, these studies have identified 21 miRNAs with increased placental expression in preeclampsia and/or pre-term birth when compared with normal pregnancies, with eight of these miRNAs showing increased expression in at least two studies (**Table 1**). Although empirical evidence is currently lacking for the targets of many C19MC miRNAs, miR-520g, and miR-520h have been shown experimentally to repress expression of *VEGF*, an angiogenic gene implicated in preeclampsia.[55] Furthermore, expression of the VEGF receptor gene, *FLT1,* has also shown consistently higher expression in placentas from preeclamptic pregnancies.[56–62] Additionally, the cell cycle inhibitor and apoptosis associated *CDKN1A* (p21) gene is a validated target of

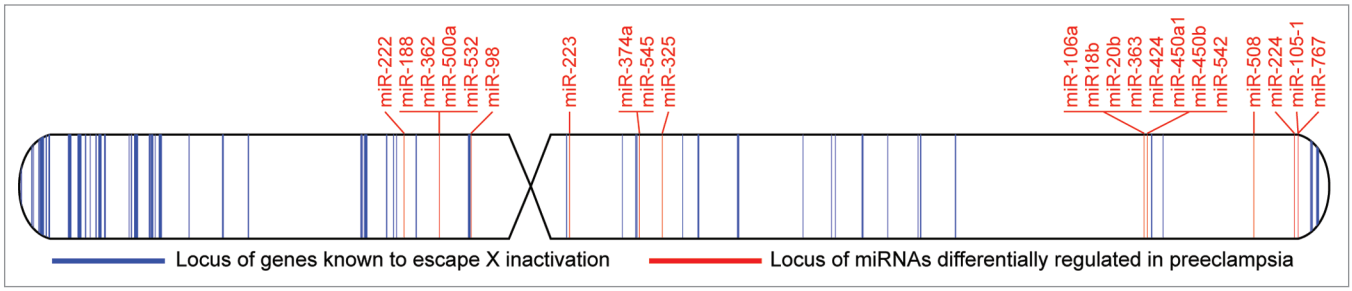

Figure 2. Idiogram representation of the human X chromosome showing the X-linked miRNAs associated with preeclampsia (red) occur in clusters in close proximity of genes that escape X inactivation (blue). The data for miRNAs showing altered expression in preeclampsia were derived from references 51–54, 103, and 108 and the data for genes that escape inactivation were adapted from reference 80 with genomic coordinates converted to hg19 coordinates using the UCSC liftOver tool.

several C19MC miRNAs differentially expressed in preeclampsia (**Table 1**), which further implicates these miRNA genes given the links preeclampsia has with perturbed apoptosis.[63]

These results are of particular interest for several reasons, particularly with respect to preeclampsia. First, as the C19MC region is imprinted, increased miRNA expression may result from loss of imprinting, and the loss of imprinting of other placental ncRNAs such as *H19* has known associations with preeclampsia.[36] Second, C19MC allelic repression is regulated by DNA methylation imprints,[45] and alteration to DNA methylation in the placenta is also associated with preeclampsia.[64] Third, at least two miRNAs in the C19MC cluster target the *VEGFA* gene that is closely networked to the *FLT1* gene. Fourthly, at least three C19MC miRNAs have been shown to target the ELAVL1 gene, which may be involved in regulating miR-675 excision from *H19* transcript (see discussion of ref. 43 above).

The differential expression of several C19MC miRNAs in the placenta is also associated with preterm labor.[65] Preterm birth and subsequent preterm delivery allows the investigation of placental gene expression at a much earlier time-point than normal labored deliveries. As such, the changes in miRNA expression may simply reflect the changing patterns of C19MC miRNAs as gestation progresses, and not be indicative of pathology. However, expression of C19MC miRNAs in cells derived from matched placentas sampled during both first trimester and term is comparable,[46] suggesting the aberrant C19MC regulation in preterm birth is not due to developmental stage differences. Together, these findings warrant further inquiry into the biological role of the C19MC miRNAs, particularly in identifying their regulatory potential, as this increased understanding could reveal novel therapeutic targets.

X-Chromosome ncRNAs in Placental Development and Pregnancy Complications

During intrauterine development, there are distinct sex differences in fetal growth trajectories and hence in birth weight,[66–68] with a sex bias in the prevalence of preterm birth,[69,70] pregnancy complications such as preeclampsia[71,72] and perinatal death.[73] As fetal growth and development are highly dependent on the exchange efficiency and capacity of the placenta, sex-specific differences in normal and pathological fetal development are most likely due to sex differences in placental function. Recent work has shown that there is a distinct male sex bias in the prevalence of placental dysfunction,[72] which supports the findings of previous studies[74–78] showing sex biases in a spectrum of pregnancy conditions and fetal health associated with abnormal placentation. However, the underlying mechanisms that predispose one sex over the other to deviate from a normal course of fetal development remain unknown.

Since the majority of genes are autosomal, many sexually dimorphic traits are driven by the sex-biased expression of autosomal genes.[79] For decades, much of the scientific literature has solely attributed the influence of sex hormones to sexual dimorphism, yet increasing evidence suggests sex chromosome genes are also implicated in the regulation of autosomal gene expression (for a review see ref. 79). While many sex-specific gene expression differences have been appreciated for some time, their phenotypic and clinical implications, particularly in the placenta and in pregnancy complications, remain relatively unexplored.[80–83]

X-Linked miRNAs as Potential Drivers of Sex Differences in Placental Gene Expression

When comparing the X chromosome to the 22 autosomes, the human X chromosome (with 140 annotated miRNAs in miRBase[84]) appears to be enriched for miRNA genes when considering its size and genomic content. Only chromosome 1, which features 11 more miRNA genes than the X chromosome, is richer in miRNA content and is ~100 MB larger in size. In contrast, the Y chromosome has only two annotated miRNA genes in a pseudo-autosomal region, which undergoes recombination with the X chromosome.

The observation that X chromosomes have high miRNA gene content highlights the potential of X-linked miRNAs to contribute to sex-biased autosomal gene expression. As X-linked miRNAs can potentially target multiple autosomal genes, sex-biased expression of X-linked miRNAs could trigger cascade-like effects, potentially driving sex-biased expression of many autosomal genes.[85] Additionally, ~35 X-linked miRNAs are located within introns of protein-coding genes which are likely to

share transcriptional elements with their host genes, potentially resulting in co-regulation. For example, the X-linked gene *CHM,* which hosts miR-361, is known to escape X chromosome inactivation (XCI), which could therefore lead to sex-biased expression of miR-361, and through autosomal gene targeting result in sex biased expression of autosomal genes.

Previous Studies Provide Few Clues to Which miRNAs Escape XCI

Although X-linked miRNAs are potential drivers of sex differences in placental development and function, there is little evidence to suggest which, if any, of these miRNAs actually escape XCI, leading to biallelic (and potentially increased) expression in females. The inactivation of X chromosomes in human extra-embryonic tissues has been a topic of continual research over the past three decades, although the status and extent of XCI in the human placenta remains controversial. Most early studies focused on allele-specific expression from the *G6PD* (Xq28) locus to determine if the X chromosome was randomly inactivated or skewed toward paternal or maternal XCI, and showed that XCI varies notably across samples, with patterns of random and skewed X-inactivation.[86–90] In later attempts to clarify the status of XCI in the human placenta, research shifted to different loci, again showing mixed results of skewed and random XCI.[91–96] Taken together, these results suggest a high degree of XCI heterogeneity in the extra-embryonic tissues of female fetuses. Although only assessing genes on the q-arm of the X chromosome, these studies suggest that when XCI deviates from random, it is the paternal X chromosome that is most often inactivated.

What has become increasingly apparent over the past decade, however, is that multiple X chromosome regions escape inactivation, and that these extend beyond chromosomal regions with Y chromosome homologs.[80,97] As the results of any XCI assay are dependent on the loci under investigation, it now appears spurious to infer the regulation of a whole chromosome (or region) based on the assessment of one or two X chromosome loci. In an attempt to widen the scope of our understanding of XCI in the human placenta, recent work has demonstrated allele-specific expression profiles of 22 genes spread across the X chromosome.[98] The results of this most comprehensive placental study to date suggest that XCI in the human placenta is random, with localized mosaic patterns of maternal and paternal XCI.[98] However, given the number of samples and the methodological limitations,[99–101] placental XCI studies still lack the depth of XCI research conducted using other human tissues. Subsequently, we have very little indication of what ncRNAs, particularly miRNAs, escape XCI and potentially contribute to sex-biased placental gene expression in normal and complicated pregnancies.

X-linked miRNAs Associated with Preeclampsia

Recent studies have shown many X chromosome miRNAs that occur in clusters are differentially regulated in placentas from preeclamptic pregnancies (**Fig. 2**). When summarizing the results of these studies, increased expression of miR-20b[53,54,102] and miR-222[54,103] and decreased expression of miR-223[52,53] is supported by two or more studies. Of particular interest, these miRNA have been shown experimentally to target multiple genes involved in processes such as apoptosis, angiogenesis and immune response (**Table 1**), all of which are implicated in the pathogenesis of preeclampsia.[63,104,105]

There is also evidence implicating the miRNA cluster at Xq26.3, which flanks the placenta-specific *PLAG1* gene. This cluster contains six miRNAs, four of which (miR-424, miR-542, miR-450a-1, and miR-450b) have been shown to be differentially regulated in placentas from preeclamptic pregnancies.[51–54] Curiously, at the individual miRNA level, miR-424 is upregulated in preeclampsia, while miR-542 and miR-450b are downregulated, and for miR-450a-1 the data are conflicting.[52,54] miR-424 is an interesting case since it overlaps the transcription start site of MGC16121, a lincRNA that is virtually unstudied. Additionally, miR-424 is a hypoxia-induced regulator of *HIF1A* and involved in angiogenesis,[106] highlighting its potential role in preeclampsia.

Although many of these miRNAs are in close proximity to genes shown to escape XCI (**Fig. 2**), sample sex information would be required to determine if these X-linked miRNA expression differences are indeed sex related and resulting from differential X chromosome epigenetic regulation. However tenuous the link between XCI and expression differences in preeclampsia, the preliminary evidence discussed here justifies further investigation. Future work focused on delineating the boundaries of XCI in the human placenta and validating the targets of miRNAs that escape XCI may provide clues to the mechanisms giving rise to sex-biases in placental development and the downstream implications for adverse pregnancy outcomes such as preeclampsia.

Conclusions

Non-coding RNAs are increasingly implicated in many developmental and pathological processes; placental development is no exception. Although much research points toward the central role of ncRNAs in placental development and function, large gaps in our knowledge remain. In particular, the ncRNAs under epigenetic regulatory control through mechanisms such as XCI and genomic imprinting appear to be influential players. However, many questions remain regarding the functional actions of these transcripts and whether their change in expression in associated pregnancy complications is a cause or consequence. In either case, increasing our understanding of the epigenetically regulated ncRNAs in normal placental development is essential if these perplexing molecules are ever to be used as diagnostic or predictive biomarkers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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