MicroRNAs: crucial regulators of placental development

Heyam Hayder, Jacob O'Brien, Uzma Nadeem and Chun Peng

Department of Biology, York University, Toronto, Ontario, Canada

Correspondence should be addressed to C Peng; Email: cpeng@yorku.ca

Abstract

MicroRNAs (miRNAs) are small non-coding single-stranded RNAs that are integral to a wide range of cellular processes mainly through the regulation of translation and mRNA stability of their target genes. The placenta is a transient organ that exists throughout gestation in mammals, facilitating nutrient and gas exchange and waste removal between the mother and the fetus. miRNAs are expressed in the placenta, and many studies have shown that miRNAs play an important role in regulating trophoblast differentiation, migration, invasion, proliferation, apoptosis, vasculogenesis/angiogenesis and cellular metabolism. In this review, we provide a brief overview of canonical and non-canonical pathways of miRNA biogenesis and mechanisms of miRNA actions. We highlight the current knowledge of the role of miRNAs in placental development. Finally, we point out several limitations of the current research and suggest future directions.

Reproduction (2018) **155** R259–R271

Introduction

MicroRNAs (miRNAs) have been established as major regulators of gene expression and are involved in many biological processes (Vasudevan 2012, Jonas & Izaurralde 2015). Since their discovery in 1993, miRNAs have been of great interest to researchers and many new advances have been made in understanding their structure, regulation and mechanisms of action (Lee *et al.* 1993, Jonas & Izaurralde 2015). Most studies have shown that miRNAs suppress gene expression when bound to the 3' untranslated region (UTR) of target mRNAs by inhibiting translation and reducing mRNA stability (Behm-Ansmant *et al.* 2006, Chen *et al.* 2010, Miao *et al.* 2016). However, additional modes of action for miRNAs, such as transcriptional regulation and activation of gene expression, have also been reported (Benhamed *et al.* 2012, Vasudevan 2012, Catalanotto *et al.* 2016, Miao *et al.* 2016).

The placenta is a transient organ essential for the survival and development of mammalian embryos (Rossant & Cross 2001). This organ plays critical roles in mediating the exchange of respiratory gases, nutrients and waste products between the mother and the fetus (Rossant & Cross 2001, Regnault *et al.* 2002, Wooding & Burton 2008). In addition, the placenta also acts as an endocrine organ and produces many pregnancy-associated hormones and growth factors that help in sustaining pregnancy, preventing fetus rejection by the mother's immune system and regulating fetal growth (Rossant & Cross 2001, Fu *et al.* 2013a, Ji *et al.* 2013).

Placental development is a spatially and temporally regulated process. This allows for increasing oxygen

and nutrient demands required by the growing fetus to be met throughout gestation (Wooding & Burton 2008). Improper placental formation gives rise to many pregnancy-associated conditions such as preeclampsia and intrauterine growth restriction (Genbacev *et al.* 1996, Rossant & Cross 2001, Fu *et al.* 2013a). In recent years, the role of miRNAs in placentation has been increasingly recognized. In this review, we aim to provide an updated summary of the role of miRNAs in regulating various trophoblast activities and placental development. Dysregulation of miRNAs and their potential involvement in pregnancy complications has been discussed recently (Fu *et al.* 2013a, Mouillet *et al.* 2015, Escudero *et al.* 2016, Cai *et al.* 2017) and therefore will not be included in this review.

Overview of microRNAs

miRNAs are endogenous, small non-coding single-stranded RNAs, on average 22 nt in length, and are involved in multiple modes of gene regulation (Truesdell *et al.* 2012, Vasudevan 2012, Havens *et al.* 2014, Valinezhad Orang *et al.* 2014, Jonas & Izaurralde 2015, Catalanotto *et al.* 2016, Xiao *et al.* 2016). miRNAs are processed post- or co-transcriptionally from RNA polymerase II/III transcripts (Ha & Kim 2014). Approximately half of all known miRNA genes are intragenic, contained mostly within the introns and relatively few exons of protein coding genes (de Rie *et al.* 2017). The remaining miRNA genes are transcribed independent of a host gene via their own promoters (Kim & Kim 2007, Fuziwara & Kimura 2015).

Figure 1 Overview of canonical microRNA biogenesis and mechanism. Canonical miRNA biogenesis is both Drosha- and Dicer-dependent. Following transcription, the primary (pri-) miRNA is identified and cleaved by the endoribonuclease, Drosha, to produce the precursor (pre-) miRNA. Nuclear export of the pre-miRNA is facilitated by the Exportin 5/RanGTP transport system. Once in the cytoplasm, the pre-miRNA is subject to terminal loop cleavage by the endoribonuclease Dicer. After cleavage, the mature miRNA duplex is loaded into the Argonaute family of proteins and the passenger strand is degraded, forming the miRNA-induced silencing complex (miRISC). The gene regulatory power of cytoplasmic miRISC typically culminates in gene silencing by mediating induction of translation inhibition, mRNA poly(A) deadenylation and mRNA degradation via interaction at the 3' untranslated region of target mRNA. After target association and following recruitment of GW182 and associated proteins into miRISC, translation initiation is inhibited, preventing nascent protein translation of the target mRNA molecule. It is hypothesized that miRISC-induced dissociation of the translation initiation complex, eIF4F, from the 5' cap of mRNA and/or its functional disruption suppresses translation initiation. Interaction of GW182 with poly(A) binding proteins (PABPC) and poly(A) deadenylase complexes PAN2/3 and CCR4-NOT localizes the 3' mRNA tail to the miRISC complex, promoting efficient target mRNA deadenylation. Complete poly(A) deadenylation leads to decapping-protein 2 (DCP2)-mediated mRNA decapping, exposing the mRNA to 5'–3' degradation via the exoribonuclease XRN1.

The vast majority of miRNAs are processed through the canonical biogenesis pathway (Kim *et al.* 2016) (Fig. 1). Canonical miRNA biogenesis begins with the

detection of the primary miRNA transcript (pri-miRNA), contained within nascent RNA, by DiGeorge Critical Region 8 (DGCR8) and associated proteins through recognition of the RNA N6-methyladenylated GGAC motif (Alarcon *et al.* 2015). In complex with DGCR8 is the nuclear RNase III endonuclease Drosha which cleaves the pri-miRNA duplex proximal to the base of the characteristic hairpin structure of pri-miRNA. This produces the excised precursor (pre-)miRNA containing a 2 nucleotide 3' overhang (Han *et al.* 2004). Together, Drosha and DGCR8 are termed the microprocessor complex (Denli *et al.* 2004).

Following pri-miRNA cleavage, the pre-miRNA is exported to the cytoplasm through an exportin 5 (XPO5)/RanGTP complex and then processed by the predominantly cytoplasmic RNase III endonuclease Dicer (Denli *et al.* 2004, Doyle *et al.* 2013). This cleavage, which removes the terminal loop, produces the mature miRNA duplex from pre-miRNA (Zhang *et al.* 2004). The labeling of the two strands of the miRNA duplex is based on the directionality of the strand in the pre-miRNA. The 5' end of the pre-miRNA hairpin contains the 5p strand and the 3' end the 3p strand (previously miRNA and miRNA*). Either the 5p or 3p strand of the miRNA duplex can be loaded into the Argonaute (AGO) family of proteins (AGO1–4 in humans) in an ATP-dependent manner (Yoda *et al.* 2010, Ha & Kim 2014); the strand that is loaded into AGO is termed the guide strand.

Several non-canonical miRNA biogenesis pathways have been elucidated (Ruby *et al.* 2007, Babiarczy *et al.* 2008, Yang & Lai 2011, Abdelfattah *et al.* 2014, Ha & Kim 2014) and grouped into two general categories: Drosha/DGCR8-independent and Dicer-independent. These non-canonical pathways take advantage of the cellular machinery already in place to produce canonical miRNA by producing Drosha, Dicer and Argonaute substrates from discrete RNA sources such as small hairpin RNAs (shRNA), small nucleolar RNAs and splicing products (Yang & Lai 2011, Castellano & Stebbing 2013, Abdelfattah *et al.* 2014). Drosha/DGCR8-independent pre-miRNAs share a common trait in which separate processing mechanisms produce products which resemble Dicer substrates. For example, mirtrons encompass the group of pre-miRNAs produced from introns during mRNA splicing. Additionally, 7-methylguanosine (m^7G)-capped pre-miRNAs are transcribed such that the nascent RNA does not need Drosha cleavage and can be directly exported from the nucleus through exportin 1 (Xie *et al.* 2013). Moreover, the m^7G cap is thought to be the cause of a strong 3p strand bias. Dicer-independent miRNAs are processed from endogenous shRNA transcripts by Drosha and may be unique in their requirement for AGO2 to complete their processing within the cytoplasm. This group of pre-miRNAs is too short to be processed by Dicer, leading to the 5' loading of the entire pre-miRNA into AGO2 (Abdelfattah *et al.* 2014). Slicing of the 3p strand and

3'–5' trimming creates a strong 5p strand bias. Although non-canonical miRNAs may elicit post-transcriptional silencing capabilities and undergo regulation independent of canonical miRNAs, the vast majority of miRNAs are processed through the canonical biogenesis pathway, requiring both Drosha and Dicer to complete their maturation (Kim *et al.* 2016). However, consistent with their canonical counterparts, these non-canonical miRNAs have been linked to various cellular programs such as proliferation, de/differentiation, immune response, neural development and cellular metabolism (Abdelfattah *et al.* 2014).

Once AGO proteins are loaded and the miRNA duplex unwound, they form the minimal miRNA-induced silencing complex (miRISC) (Kawamata & Tomari 2010, Fabian & Sonenberg 2012). miRISC gains target specificity by recognition of miRNA response elements (MRE) on target RNA molecules, while the degree of complementarity determines, to some extent, the mode of regulation, i.e. direct or indirect gene silencing (Ameres *et al.* 2007, Jonas & Izaurralde 2015). A fully complementary miRNA:MRE promotes AGO2 endonuclease activity and cleavage of the target RNA molecule (Ameres *et al.* 2007). In turn, this also has the consequence of decreased miRNA stability as exact matches promote not only target cleavage but also degradation of the guide miRNA, although the mechanism is not well understood (Ameres & Zamore 2013). What is known is that the guide miRNA must first undergo the 3' addition of adenosine or uracil which promotes 3'–5' exonuclease activity, resulting in guide miRNA degradation (Krutzfeldt *et al.* 2005, Ameres *et al.* 2010).

In humans, the frequency of exact matches on target mRNA is rare (Jonas & Izaurralde 2015). The majority of validated MREs contain at least central mismatches to their guide miRNA, preventing AGO2 nuclease activity. As a consequence, AGO2 shifts from RNAi effector to mediator, and along with the non-endonucleolytic AGO family members act to recruit other proteins associated with mRNA stability. This has led to the detection of the miRNA seed region (nucleotides 2–8) that are crucial for many but not all miRNA:MRE interactions (Ellwanger *et al.* 2011, Xu *et al.* 2014, Miao *et al.* 2016). In most cases, miRNAs interact with the 3' UTR of target mRNAs, resulting in translation inhibition and mRNA deadenylation and decapping (Huntzinger & Izaurralde 2011, Fabian & Sonenberg 2012, Meijer *et al.* 2013, Ipsaro & Joshua-Tor 2015).

To form an miRISC complex capable of post-transcriptional gene silencing, mRNA-bound miRISC recruits the GW182 family of proteins which acts as a scaffold to further recruit effector protein complexes (Behm-Ansmant *et al.* 2006). Both the PAN2–PAN3 and CCR4–NOT deadenylase complexes are recruited through the unstructured, tryptophan (W) repeats of GW182 (Christie *et al.* 2013, Jonas & Izaurralde

2015). PAN2–PAN3 initially catalyzes target mRNA poly(A) deadenylation which is promoted through the interaction of W-repeats to poly(A)-binding proteins (PABPC), bringing both the mRNA poly(A) tail and deadenylase into close proximity (Jonas & Izaurralde 2015). The CCR4–NOT complex completes the deadenylation process and is followed by mRNA decapping facilitated by decapping protein 2 (DCP2) and associated proteins (Behm-Ansmant *et al.* 2006). Decapped and deadenylated mRNA are then degraded from the 5' end by the 5'–3' exoribonuclease 1 (XRN1) (Braun *et al.* 2012) (Fig. 1).

While most miRNA studies focus on how miRNAs target mRNAs by binding to MREs at the 3' UTR to suppress their expression, MREs have also been reported in the 5' UTR. miRISC interactions within the 5' UTR have been shown to both promote and suppress translation through mRNA-specific mechanisms, discussed in detail in Vasudevan (2012) and Valinezhad Orang *et al.* (2014). Moreover, cell-state-specific miRNA-mediated translational activation has been observed in human quiescent cells where nuclear AGO2 complexes with Fragile-x-mental-retardation-related protein 1 (FXR1) instead of GW182 (Truesdell *et al.* 2012). This complex was found to interact with nuclear mRNA targets which in turn led to translational activation following export to the cytoplasm (Truesdell *et al.* 2012).

Overview of placental development

Soon after fertilization, asymmetric cell division of the blastomere gives rise to different cell populations, an outer cell layer surrounding an inner cell population (Johnson & Ziomek 1981, Viswanathan *et al.* 2009). The blastocyst is formed when the outer cell layer differentiates into a layer of trophoblasts termed the trophectoderm (TE) and the inner cell population differentiates into the inner cell mass (ICM). The TE will later give rise to the placenta, while the ICM will develop into the embryo and the visceral endoderm (yolk sac) (Viswanathan *et al.* 2009, Maltepe & Fisher 2015).

With the trophectoderm formed, the blastocyst is ready for implantation (Caniggia *et al.* 2000). Implantation starts with the adhesion of the TE onto the receptive decidualized endometrium through a complex network of cell–cell communication events (Red-Horse *et al.* 2004). This leads to the invasion of the blastocyst through the extracellular matrix of the decidua by the proliferating and differentiating trophectoderm layer, embedding it deep into the uterine wall (Red-Horse *et al.* 2004, Noris *et al.* 2005, Wooding & Burton 2008).

Once the blastocyst is embedded within the uterine wall, the process of placenta formation, termed placentation, begins with the differentiation of the TE cells into the different trophoblast lineages (Red-Horse *et al.* 2004, Maltepe & Fisher 2015). Placentation in eutherian mammals is more complex compared to

marsupial mammals (Moffett & Loke 2006, Carter 2007, Maltepe & Fisher 2015). Moreover, among eutherian mammals, placentation varies considerably in the degree of trophoblast invasiveness from minimal invasion occurring in epitheliochorial placentation (e.g. pigs and sheep), intermediate invasion in endotheliochorial placentation (e.g. dogs and cats) and maximal invasion in hemochorial placentation (e.g. humans and rodents) (Moffett & Loke 2006, Carter 2007, Wooding & Burton 2008).

In humans, placentation consists, in part, of the differentiation and proliferation of the TE to form a branching network of villi that are in direct contact with the maternal circulation while simultaneously maintaining a barrier between the fetal and maternal blood (Kaufmann *et al.* 2004, Wooding & Burton 2008, Schmidt *et al.* 2015). The villi are the functional units of the placenta. They facilitate and respond to the demands of the developing fetus by regulating the exchange of gases, nutrients and wastes through the villus core, which consists of the mesenchyme and fetal blood vessels (Kaufmann *et al.* 2004). The tips of the branching villous network that come into direct contact with the endometrium are termed the anchoring villi, while the remaining villi, which float freely in the blood-filled intervillous space, are called the floating villi (Maltepe *et al.* 2010).

The highly proliferative, undifferentiated cytotrophoblast (CTB) progenitor cells of the placental villi differentiate into two general pathways. CTBs can either fuse to form a multinucleated monolayer of syncytiotrophoblasts (STBs) that enclose the villous stroma, or differentiate into invasive extravillous trophoblasts (EVTs) that infiltrate the endometrium and a portion of the myometrium (Cartwright *et al.* 2010). STBs function as a barrier, or more precisely, as an interface between fetal and maternal blood as well as in the production of pregnancy-associated hormones and growth factors important for placental and fetal development and growth (Fu *et al.* 2013a). The mechanisms that facilitate CTB fusion and production of the STB layer are still under investigation; however, formation of gap junctions, activation of apoptotic pathways and the expression of endogenous retroviral proteins such as syncytin appear to be key mechanisms (Wooding & Burton 2008).

In the EVT pathway, the proliferating CTBs of the anchoring villi form a column that attaches to the uterine epithelium and subsequently differentiates into interstitial EVT (iEVT). Interstitial EVTs (iEVTs) invade the decidua and one-third of the myometrium where they further differentiate into the multinucleated placental bed giant cells (Fu *et al.* 2013a). Endovascular EVTs (enEVTs) acquire endothelial-like characteristics and invade the maternal spiral arteries to replace the endothelial cells. This results in the transformation of spiral arteries into distended,

thin-walled vessels to ensure continuous maternal blood flow to the placenta and to maintain sufficient oxygen and nutrient supplies for the growing embryo (Anin *et al.* 2004, Lyall *et al.* 2013). Recently, endoglandular EVTs (egEVT) have been identified as a potential third subtype of EVTs (Moser *et al.* 2010, 2015). Initial findings suggest that egEVT disintegrate uterine glands and open the gland lumen to the intervillous space releasing glandular secretions that may impact placentation (Burton *et al.* 2007, Moser *et al.* 2015).

Many studies on human placental development, including the miRNAs work discussed in the following sections, have been carried out using *in vitro* models, such as immortalized trophoblast and choriocarcinoma cell lines, primary cultures of trophoblasts and/or villous explants from first trimester placenta. Rodents, especially mice, have also been used as a model. It is important to recognize that each of these models have pros and cons. Although cell lines are easy to work with, especially with respect to transient and stable transfection of genes, there are significant differences in gene expression signatures between cell lines and primary trophoblasts (Bilban *et al.* 2010). For example, chromosome 19 miRNA cluster (C19MC) members are not expressed in HTR8/SVneo cells, while chromosome 14 miRNA cluster (C14MC) members cannot be detected in JEG-3 cells (Mouillet *et al.* 2011, Morales-Prieto *et al.* 2014). Primary CTBs have been used mainly to study the differentiation of CTB to STB, but these cells have a limited life span and can only be used to study the short-term effects of transiently transfected miRNAs. Villous explants maintain the cellular architecture and mimic more closely the *in vivo* environment (Miller *et al.* 2005). However, only the short-term effect of miRNA overexpression or inhibition can be examined. The mouse model provides some insights into the *in vivo* functions of miRNAs, but it should be noted that there are significant differences between the mouse and human placentation that can affect the transferability of findings to humans (Wildman *et al.* 2006, Carter 2007, Maltepe & Fisher 2015, Schmidt *et al.* 2015, Grigsby 2016). For example, trophoblast invasion during early mouse placentation is shallow as it only extends into the decidua, whereas in humans, it proceeds to the myometrium (Carter 2007, Maltepe & Fisher 2015, Schmidt *et al.* 2015). Also, mouse trophoblasts express major histocompatibility complex (MHC)-K, -D and -L, while human trophoblasts express human leukocyte antigen G (HLA-G) or HLA-C. This leads to different interaction dynamics between uterine immune cells and invading trophoblasts (Chaouat & Clark 2015, Schmidt *et al.* 2015). Importantly, there are different miRNA expression profiles between human and mouse placentas. Specifically, C19MC is expressed only in primates with no orthologs found in rodents (Morales-Prieto *et al.* 2014), while miRNAs of the *Simt2* cluster are rodent-specific (Zheng *et al.* 2011, Schmidt *et al.*

2015, Inoue *et al.* 2017). Also, C14MC in humans shows a divergence in rodents where it is located on chromosome 12 and lacks multiple members found in humans (Seitz *et al.* 2004). Therefore, in the following discussion, we will point out which model(s) was used in each study.

miRNAs in trophoderm development and implantation

Many studies carried out in mice suggest that miRNAs play a role in trophoderm development. Examination of mouse miRNA expression patterns during trophoderm specification has revealed let-7, miR-21, miR-29c, miR-96, miR-125a, miR-214, miR-297, miR-376a and miR-424 as candidates that may play a role in this process (Viswanathan *et al.* 2009, Nosi *et al.* 2017). In mouse embryonic stem cells (ESC), overexpression of miR-15b, miR-322 and miR-467 suppressed their embryonic fate and led to the induction of a trophoblast stem-cell (TSC)-like phenotype. Further analysis revealed that these miRNAs target transcription factors *Sall1*, *Sall4*, *Pou5f1* and *Nanog* (Nosi *et al.* 2017), that are important for the maintenance of ESC self-renewal and pluripotency. In addition, the miR-302/367 cluster was found to promote TE differentiation in humans by targeting bone morphogenetic protein (BMP) inhibitors *TOB2*, *DAZAP2* and *SLAIN1* (Lipchina *et al.* 2011); BMP4 is a member of the transforming growth factor beta (TGFB) superfamily and is involved in promoting TE differentiation (Xu *et al.* 2002, Wu *et al.* 2008). In a human pulmonary artery cell line, miR-302 was also shown to target BMP4 receptor 2, while BMP signaling led to the transcriptional downregulation of the miRNA-302/367 gene cluster (Kang *et al.* 2012), which if it also occurs in trophoblasts could create an interesting signal-buffering dynamic.

Limited evidence obtained so far has suggested that miRNAs play a role in regulating implantation. First, studies in mice have shown that miRNAs are differentially expressed between implantation sites and inter-implantation sites in the endometrium (Chakrabarty *et al.* 2007, Hu *et al.* 2008, Geng *et al.* 2014). Further studies revealed that overexpression of miR-145 impaired the attachment of mouse embryos to endometrial epithelial cells by targeting insulin-like growth factor 1 receptor (*Igf1r*) (Kang *et al.* 2015). Finally, Dicer knockdown in mouse blastocysts altered miRNAs expression and resulted in a lower implantation rate (Cheong *et al.* 2014). In humans, a number of miRNAs in the endometrium, including miR-145, were also found to be differentially expressed between women who repeatedly fail to have successful implantation and fertile women (Revel *et al.* 2011). These findings suggest a possible role of miRNAs in regulating implantation;

however, more studies are required to understand the functions of miRNAs and their underlying mechanisms in this process.

Another important aspect of successful implantation is the interaction between the fetal blastocyst and the maternal immune cells. Early in pregnancy, maternal uterine natural killer (uNK) cells, T cells, B cells, macrophages and dendritic cells are recruited into the endometrium at the site of implantation to help regulate placental and fetal development (Szekeres-Bartho 2002, Bidarimath *et al.* 2014, Zhang *et al.* 2016a). As mentioned earlier, human EVT expresses a limited variety of MHC molecules, mostly HLA-G and HLA-C (Bidarimath *et al.* 2014, Schmidt *et al.* 2015, Hackmon *et al.* 2017). HLA-G interacts with the maternal killer immunoglobulin-like receptors expressed by uNK cells, resulting in the activation of uNK cytokine production but not its cytotoxicity response (Rajagopalan *et al.* 2006). This in turn promotes maternal immunological tolerance and placental development and vascularization (Bidarimath *et al.* 2014, Ratsep *et al.* 2015). Both miR-148a and miR-152 were found to bind the 3' UTR of *HLA-G*, amplified from the JEG-3 human trophoblast cell line, downregulating its expression and thereby reducing HLA-G mediated inhibition of natural killer cells cytotoxicity (Manaster *et al.* 2012). These findings suggest that miRNAs play a role in regulating maternal immunological tolerance to invading EVT. In addition, miRNAs have also been shown to help regulate other maternal immune cells such as macrophages, endometrial dendritic cells and T cells in the pregnant uterus and have been extensively reviewed in Robertson and Moldenhauer (2014), Mori *et al.* (2016), Schjenken *et al.* (2016) and Robertson *et al.* (2017).

Interestingly, miRNAs were also shown to promote antiviral immunity in both trophoblast and non-trophoblast cells. In alignment with the role of placenta to protect the developing fetus, trophoblasts are the first line of defense against external factors that can impair fetal development. Therefore, it is not surprising that primary human trophoblasts are highly resistant to viral infection (Delorme-Axford *et al.* 2013). More importantly, they can confer this resistance to other types of cells when these cells uptake exosomes naturally secreted by primary trophoblasts; the exosomes were found to contain members of C19MC, miR-512-3p, miR-516b-5p and miR-517-3p (Bayer *et al.* 2015). These C19MC miRNAs initiated autophagy in recipient cells without leading to cell death which was suggested to impair viral replicability (Delorme-Axford *et al.* 2013, Bayer *et al.* 2015). Thus, miRNAs play a dynamic role to not only promote decidual immune tolerance in support of the growing fetus but also protect both mother and fetus from viral infection (Mouillet *et al.* 2014, Ouyang *et al.* 2014).

miRNAs in trophoblast differentiation, migration and invasion

Several studies have suggested that miRNAs are important regulators of CTB to STB differentiation. Microarray analyses of miRNA expression profiles in primary trophoblast before and after their differentiation into STB have revealed that multiple members of C19MC such as miR-515-5p, miR-518f, miR-519c-3p and miR-519e-5p were significantly downregulated during CTB to STB differentiation (Zhang *et al.* 2016b). Further investigation showed that miR-515-5p targeted several genes that play critical roles in STB differentiation, including human glial cell missing-1 (*GCM1*) (Yu *et al.* 2002, Liang *et al.* 2010, Wakeland *et al.* 2017) and frizzled 5 (*FZD5*) (Lu *et al.* 2013) and significantly reduced cell fusion (Zhang *et al.* 2016b). Another miRNA gene cluster is the miR-17–92 family that is located on chromosome 13 and encodes six miRNAs (miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a and miR-92a) (Concepcion *et al.* 2012). Multiple members of the miRNA-17–92 cluster, and its paralog cluster miR-106a–363, have been found to silence *GCM1* in primary cultures of human trophoblasts. These miRNAs are downregulated during CTB to STB differentiation, thereby promoting the differentiation process (Kumar *et al.* 2013). Studies from our laboratory have demonstrated that miR-378a-5p suppressed BeWo cell fusion and STB marker gene expression by targeting cyclin G2 (*CCNG2*), suggesting that it inhibits STB differentiation (Nadeem *et al.* 2014).

Many studies have reported that miRNAs regulate EVT differentiation, migration and invasion by targeting key pathways known to regulate these processes. Early placental development occurs in a hypoxic environment, and oxygen tension has been reported to regulate many cellular processes in the placenta, including proliferation, EVT differentiation and invasion (Chang *et al.* 2018). However, the precise role of oxygen tension in EVT differentiation and invasion is still not well understood. Earlier studies have suggested that hypoxic conditions during early pregnancy are in part responsible for the high rate of trophoblast proliferation and inhibition of EVT invasion (Red-Horse *et al.* 2004). As the trophoblasts invade deeper into the uterus, where oxygen levels are higher, they shift from a more proliferative phenotype to a more migratory and invasive phenotype (Genbacev *et al.* 1997, Kaufmann & Castellucci 1997, Knofler 2010). However, hypoxia was recently found to promote EVT differentiation in a hypoxia-inducible factor (HIF)-dependent manner while inhibiting STB differentiation in primary cultures of human CTB (Wakeland *et al.* 2017). Thus, it is proposed that low oxygen induces the differentiation into immature EVT, but further maturation of EVT and invasion increase with rising oxygen tension (Chang *et al.* 2018).

Since hypoxia plays an important role in early placental development, studies have investigated its

effects on miRNA expression and function (Donker *et al.* 2007, Mouillet *et al.* 2010, Fu *et al.* 2013a). They have revealed a group of miRNAs that are upregulated under hypoxia, a subset of which, hypoxamirs, are under direct regulation of hypoxia-induced transcription factors (Kulshreshtha *et al.* 2007). MiR-210 is the most well-studied example of hypoxamirs, upregulated directly by HIF1A (Camps *et al.* 2008); additionally, it is regulated by a hypoxia-responsive transcription factor, nuclear factor kappa-B subunit p50 (NFKB1), in primary human trophoblasts (Zhang *et al.* 2012). It was reported that miR-210 inhibited migration and invasion in primary CTBs (Zhang *et al.* 2012), HTR8/SVneo cell line (Luo *et al.* 2016), and primary EVT (Anton *et al.* 2013) by targeting ephrin-A3 (*EFNA3*), homeobox-A9 (*HOXA9*) (Zhang *et al.* 2012), and thrombospondin type I domain containing 7A (*THSD7A*) (Luo *et al.* 2016) or by activating the MAPK pathway (Anton *et al.* 2013). However, knockout of miR-210 did not result in significant changes in fetal or placental weight and non-severe hypoxia (12% O₂) did not increase miR-210 in these mice, suggesting that miR-210 may be dispensable for fetal-placental development under normoxic and non-severe hypoxic conditions (Krawczynski *et al.* 2016). Thus, the role of miR-210 in hypoxia-regulated placental development requires further investigation.

miRNAs also regulate EVT differentiation and invasion by modulating growth factor signaling. An important family of growth factors in placental development is the TGFB superfamily. Many miRNAs have been found to enhance EVT migration and invasion by targeting members of the TGFB family. For example, miR-376c targeted both activin receptor-like kinase 7 (*ALK7*) and *ALK5* to impede TGFB/Nodal signaling (Fu *et al.* 2013b), while miR-378a-5p targeted the ligand Nodal (Luo *et al.* 2012) to promote migration and invasion in HTR8/SVneo cells and EVT outgrowth in first trimester placental villous explants. Similarly, miR-195 enhanced trophoblast invasion by targeting activin receptor type-2B, a type II receptor for Nodal and activin, in HTR8/SVneo cells (Wu *et al.* 2016).

Using HTR8/SVneo, JEG-3 or BeWo trophoblast cell lines, several studies have suggested that miRNAs also regulate EVT motility by targeting other genes involved in regulating cell invasion. Both miR-346 and miR-582-3p targeted endocrine-gland-derived vascular endothelial growth factor (*EG-VEGF*) as well as matrix metalloproteinase 2 (*MMP2*) and *MMP9*, and strongly inhibited the migratory and invasive abilities of trophoblasts (Su *et al.* 2017). Similarly, miR-93 (Pan *et al.* 2017) and miR-204 (Yu *et al.* 2015), which targeted *MMP2* and *MMP9*, respectively, inhibit cell invasion. Members of the C19MC, miR-519d-3p (Ding *et al.* 2015) and miR-520g (Jiang *et al.* 2017a) also targeted *MMP2* and inhibited migration and invasion, while miR-520c-3p inhibited invasion by suppressing *CD44*, which is needed for the interaction between EVTs and decidual

extracellular matrix (Takahashi *et al.* 2017). On the other hand, miR-21 promoted not only migration and invasion but also cell proliferation (Chaiwangyen *et al.* 2015). Among its targets is phosphatase and tensin homolog (*PTEN*), a known inhibitor of the AKT pathway. *PTEN* dephosphorylates phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P₃), leading to inactivation of AKT which is involved in trophoblast cell motility (Chaiwangyen *et al.* 2015). MiR-34a inhibited invasion by targeting *MYC* (Sun *et al.* 2015). MiR-20a is another such miRNA where it inhibited not only trophoblast motility but also cell proliferation by targeting forkhead box protein A1 (*FOXA1*) (Wang *et al.* 2014). As all these studies were only done in cell lines, the significance of these miRNAs in EVT differentiation and invasion requires validation using additional model systems.

miRNAs in trophoblast proliferation and apoptosis

Proliferation and apoptosis are important mechanisms of proper placental development; disruption of the equilibrium between cell division and death impairs placental function (Levy *et al.* 2000). A recent *in vivo* study in mice has demonstrated the critical role of the miR-290 cluster in placental cell proliferation and placental growth; deletion of the miR-290 cluster resulted in the reduction of trophoblast progenitor cell proliferation and placental size (Paikari *et al.* 2017). In addition, many *in vitro* studies have shown that miRNAs regulate trophoblast proliferation and apoptosis. For example, miR-378a-5p (Luo *et al.* 2012) and miR-376c (Fu *et al.* 2013b) enhanced HTR8/SVneo cell proliferation and survival and EVT outgrowth in villous explants by inhibiting Nodal/TGFB signaling. On the other hand, miR-195 inhibited apoptosis through targeting of inducible nitric oxide synthase (*iNOS*) in HTR8/SVneo cells (Wang *et al.* 2017). Furthermore, overexpression of miR-377 and let-7a, which are upregulated in term placenta samples versus first trimester samples, decreased trophoblast proliferation by reducing ERK and/or *MYC* expression in first trimester placental explants (Farrokhnia *et al.* 2014). Together, these studies suggest a potential regulatory link between miRNAs and proliferation in human trophoblasts.

Studies using multiple human trophoblast cell lines suggested a role of miRNAs in the regulation of apoptosis. The miR-29 family (miR-29a/b/c) promoted apoptosis by targeting myeloid cell leukemia-1 (*MCL1*), an apoptosis regulator and a member of the BCL2 family (Li *et al.* 2013, Gu *et al.* 2016). Overexpression of miR-18a increased apoptosis by inducing the expression of estrogen receptor alpha (*ESR1*) (Zhu *et al.* 2015), while miR-128a induced apoptosis via the mitochondrial pathway by downregulating *BAX* (Ding *et al.* 2016) and miR-30a-3p by inhibiting *IGF1* (Niu *et al.* 2018). On the other hand, miR-101 targeted endoplasmic reticulum protein 44 (*ERP44*) to suppress ER-stress-induced

apoptosis (Zou *et al.* 2014). Again, as majority of these studies were carried out using only cell lines, more studies are required to confirm the involvement of these miRNAs in trophoblast cell proliferation and apoptosis.

miRNAs in placental vascular development

Placenta vascularization is essential to meet the metabolic demands of the rapidly growing fetus. Delayed or reduced vascular development of the placenta can result in compromised pregnancies (Reynolds & Redmer 2001). Placental vascular formation includes vasculogenesis, the *de novo* synthesis of vessels within the villi core and angiogenesis, the formation of new vessels from preexisting ones (Huppertz & Peeters 2005, Demir *et al.* 2007). Recently, deletion of the miR-290 cluster in mice has been reported to cause disorganization of the vasculature in the labyrinth (Paikari *et al.* 2017), providing strong evidence that miRNAs are important regulators of placenta vascular development.

Several miRNAs have also been suggested to play a role in vasculogenesis and angiogenesis. It was reported that miR-126 promotes proliferation, differentiation and migration of human endothelial progenitor cells by targeting an anti-angiogenic factor *PIK3R2* (Yan *et al.* 2013). Also, in pregnant rats, miR-126 was found to increase vascular sprouting, as well as placental and fetal weights (Yan *et al.* 2013). The importance of miR-126 in placenta vascular development is further supported by the finding that downregulation of miR-126 contributes to endothelial dysfunction (Yan *et al.* 2013).

VEGF is a highly regulated pro-angiogenic factor known to initiate vasculogenesis in the placenta, induce endothelial cell proliferation and migration and inhibit apoptosis (Wang & Zhao 2010). Several miRNAs have been reported to target *VEGF*. For example, miR-16 directly targeted *VEGF* to inhibit HUVEC proliferation, migration and tube formation (Zhu *et al.* 2016). Also, overexpressing miR-16 in mice placentas decreased placental and fetal weights and inhibited the total placental vasculature and capillary number (Zhu *et al.* 2016). Similarly, miR-136 (Ji *et al.* 2017), miR-200c, -20a and -20b (Hu *et al.* 2016) also targeted *VEGF*, and may exert inhibitory effects on angiogenesis. However, whether these miRNAs affect placental vascular development has not been investigated. In CD34+ endothelial cells isolated from human umbilical cord blood, miR-210 was induced by VEGF and exerted proangiogenic effects (Alaiti *et al.* 2012), suggesting that miR-210 may play a role in placental angiogenesis.

miRNAs in trophoblast cellular metabolism

Early in pregnancy, and before spiral artery plug dissolution, placental and fetal nutrients and oxygen supply is dependent on endometrial secretions and maternal plasma (Murray 2012). As a consequence,

first trimester placenta has a relatively low oxygen concentration (1–3%) (Pringle *et al.* 2010, Murray 2012) and placental cells use glycolysis and lactic acid fermentation for ATP synthesis as their primary metabolic fuel source to conserve oxygen supplies for fetal tissues (Murray 2012, Kolahi *et al.* 2017). Moreover, HIF1A downregulates mitochondrial oxygen consumption (Papandreou *et al.* 2006) to reduce ROS production at complex 3 of the electron transport chain (ETC) in the mitochondria (Colleoni *et al.* 2013). The hypoxia-induced miR-210 has been reported to regulate cellular metabolism. Using primary human trophoblasts, it was found that overexpression of miR-210 reduced, while inhibition of miR-210 increased, mitochondrial respiration (Muralimanoharan *et al.* 2012). Iron-sulfur complex assembly proteins (ISCU) and cytochrome-c oxidase assembly protein (COX10), which play important roles in the mitochondria ETC and tricarboxylic acid cycle, have been shown to be targeted by miR-210 in human endothelial and cancer cell lines (Chan *et al.* 2009, Chen *et al.* 2010). In trophoblasts, miR-210 was also found to directly target *ISCU* and to reduce the expression of *ISCU* and *COX10* (Muralimanoharan *et al.* 2012, Colleoni *et al.* 2013), suggesting that these genes are involved in miR-210-regulated trophoblast mitochondrial adaptation to low oxygen.

In addition to miR-210, several other miRNAs are also involved in mitochondrial biogenesis and function. For example, miR-130b-3p was found to decrease signals for mitochondrial biogenesis and adaptation to oxidative stress through targeting of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC1A*), a major regulator of mitochondrial

biogenesis and energy metabolism (Jiang *et al.* 2017b). Also, miR-143 overexpression in primary human trophoblasts upregulated mitochondrial complexes 1, 2 and 3 but not 4 and 5 (Muralimanoharan *et al.* 2016), thus improving mitochondrial function. It also targeted hexokinase-2, a rate-limiting enzyme of glycolysis, and as a result reduced the glycolysis rate in trophoblasts (Muralimanoharan *et al.* 2016). Together, these miRNAs may help regulate trophoblast metabolic adaptation to change in oxygen levels throughout gestation.

Concluding remarks

The placenta is an essential organ for pregnancy. The proper development of placenta requires precise regulation by many signaling molecules, including miRNAs. Increasing evidence suggests that miRNAs play important roles in regulating many key processes in placental development, such as trophoblast differentiation, migration, invasion, proliferation, apoptosis, vasculogenesis/angiogenesis and cellular metabolism (Fig. 2). Although several recent *in vivo* studies in animal models have provided strong evidence that miRNAs are critical regulators of placental development (Ito *et al.* 2015, Zhu *et al.* 2016, Paikari *et al.* 2017), there are differences in placental development and placental miRNA expression profiles between mice and humans. Therefore, applications of findings from different animal models into humans should be treated with caution. Furthermore, most reported miRNA studies in placenta were performed using human cell lines derived from immortalized first trimester trophoblasts or choriocarcinoma, while only a

Figure 2 MicroRNAs involved in placental development. Proper development and functioning of the placenta requires precise control of trophoblast proliferation, apoptosis, differentiation, cellular metabolism, as well as vasculogenesis/angiogenesis. Many miRNAs have been suggested to play a regulatory role in one or more of these processes and are listed in this Venn diagram.

smaller proportion of studies used primary cultures of trophoblasts, placental explants and/or clinical samples. There are also reports of differential miRNA expression patterns between primary cells and immortalized trophoblast cell lines. Therefore, the use of multiple model systems should be emphasized.

Most studies conducted today focus on one or a few target genes. Since miRNAs target many genes, the use of multi-omics approaches to investigate gene networks responsible for the regulatory functions of miRNAs in the placenta will provide a better understanding of how miRNAs are involved in regulating placental development. Finally, all miRNA studies in placenta focused on canonical 3' UTR-mediated gene silencing. As our understanding of the different miRNA biogenesis pathways and modes of miRNA action continues to expand, their novel contributions to modulating cellular activities during pregnancy should also be investigated.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

Work in our laboratory was supported by grants from the Canadian Institutes of Health Research (MOP-81370, CCI-92222, CCI-132565 and PJT-153146) to C P H H and J O were supported by the Ontario Graduate Scholarship.

Acknowledgement

We would like to thank Dr Jelena Brkic for critical reading and editing of the manuscript.

References

- Abdelfattah AM, Park C & Choi MY 2014 Update on non-canonical microRNAs. *Biomolecular Concepts* **5** 275–287. (<https://doi.org/10.1515/bmc-2014-0012>)
- Alaiti MA, Ishikawa M, Masuda H, Simon DI, Jain MK, Asahara T & Costa MA 2012 Up-regulation of miR-210 by vascular endothelial growth factor in ex vivo expanded CD34+ cells enhances cell-mediated angiogenesis. *Journal of Cellular and Molecular Medicine* **16** 2413–2421. (<https://doi.org/10.1111/j.1582-4934.2012.01557.x>)
- Alarcon CR, Lee H, Goodarzi H, Halberg N & Tavazoie SF 2015 N6-methyladenosine marks primary microRNAs for processing. *Nature* **519** 482–485. (<https://doi.org/10.1038/nature14281>)
- Ameres SL & Zamore PD 2013 Diversifying microRNA sequence and function. *Nature Reviews Molecular Cell Biology* **14** 475–488. (<https://doi.org/10.1038/nrm3611>)
- Ameres S, Martinez J & Schroeder R 2007 Molecular basis for target RNA recognition and cleavage by human RISC. *Cell* **130** 101–112. (<https://doi.org/10.1016/j.cell.2007.04.037>)
- Ameres SL, Horwich MD, Hung JH, Xu J, Ghildiyal M, Weng Z & Zamore PD 2010 Target RNA-directed trimming and tailing of small silencing RNAs. *Science* **328** 1534–1539. (<https://doi.org/10.1126/science.1187058>)
- Anin SR, Vince G & Quenby S 2004 Trophoblast invasion. *Human Fertility* **7** 169–174. (<https://doi.org/10.1080/14647270400006911>)
- Anton L, Olarerin-George AO, Schwartz N, Srinivas S, Bastek J, Hogenesch JB & Elovitz MA 2013 miR-210 inhibits trophoblast invasion and is a serum biomarker for preeclampsia. *American Journal of Pathology* **183** 1437–1445. (<https://doi.org/10.1016/j.ajpath.2013.07.021>)
- Babiarz JE, Ruby JG, Wang Y, Bartel DP & Blelloch R 2008 Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. *Genes and Development* **22** 2773–2785. (<https://doi.org/10.1101/gad.1705308>)
- Bayer A, Delorme-Axford E, Sleighter C, Frey TK, Trobaugh DW, Klimstra WB, Emert-Sedlak LA, Smithgall TE, Kinchington PR, Vadia S *et al.* 2015 Human trophoblasts confer resistance to viruses implicated in perinatal infection. *American Journal of Obstetrics and Gynecology* **212** 71.e1–71.e8. (<https://doi.org/10.1016/j.ajog.2014.07.060>)
- Behm-Ansmant I, Rehwinkel J, Doerks T, Stark A, Bork P & Izaurralde E 2006 MRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes and Development* **20** 1885–1898. (<https://doi.org/10.1101/gad.1424106>)
- Benhamed M, Herbig U, Ye T, Dejean A & Bischof O 2012 Senescence is an endogenous trigger for microRNA-directed transcriptional gene silencing in human cells. *Nature Cell Biology* **14** 266–275. (<https://doi.org/10.1038/ncb2443>)
- Bidarimath M, Khalaj K, Wessels JM & Tayade C 2014 MicroRNAs, immune cells and pregnancy. *Cellular and Molecular Immunology* **11** 538–547. (<https://doi.org/10.1038/cmi.2014.45>)
- Bilban M, Tauber S, Haslinger P, Pollheimer J, Saleh L, Pehamberger H, Wagner O & Knofler M 2010 Trophoblast invasion: assessment of cellular models using gene expression signatures. *Placenta* **31** 989–996. (<https://doi.org/10.1016/j.placenta.2010.08.011>)
- Braun JE, Truffault V, Boland A, Huntzinger E, Chang CT, Haas G, Weichenrieder O, Coles M & Izaurralde E 2012 A direct interaction between DCP1 and XRN1 couples mRNA decapping to 5' exonucleolytic degradation. *Nature Structural and Molecular Biology* **19** 1324–1331. (<https://doi.org/10.1038/nsmb.2413>)
- Burton GJ, Jauniaux E & Charnock-Jones DS 2007 Human early placental development: potential roles of the endometrial glands. *Placenta* **28** (Supplement A) S64–S69. (<https://doi.org/10.1016/j.placenta.2007.01.007>)
- Cai M, Kolluru GK & Ahmed A 2017 Small molecule, big prospects: microRNA in pregnancy and its complications. *Journal of Pregnancy* **2017** 6972732. (<https://doi.org/10.1155/2017/6972732>)
- Camps C, Buffa FM, Colella S, Moore J, Sotiriou C, Sheldon H, Harris AL, Gleadow JM & Ragoussis J 2008 hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. *Clinical Cancer Research* **14** 1340–1348. (<https://doi.org/10.1158/1078-0432.CCR-07-1755>)
- Caniggia I, Winter J, Lye SJ & Post M 2000 Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia. *Placenta* **21** S25–S30. (<https://doi.org/10.1053/plac.1999.0522>)
- Carter AM 2007 Animal models of human placentation – a review. *Placenta* **28** (Supplement A) S41–S47. (<https://doi.org/10.1016/j.placenta.2006.11.002>)
- Cartwright JE, Fraser R, Leslie K, Wallace AE & James JL 2010 Remodelling at the maternal-fetal interface: relevance to human pregnancy disorders. *Reproduction* **140** 803–813. (<https://doi.org/10.1530/REP-10-0294>)
- Castellano L & Stebbing J 2013 Deep sequencing of small RNAs identifies canonical and non-canonical miRNA and endogenous siRNAs in mammalian somatic tissues. *Nucleic Acids Research* **41** 3339–3351. (<https://doi.org/10.1093/nar/gks1474>)
- Catalanotto C, Cogoni C & Zardo G 2016 MicroRNA in control of gene expression: an overview of nuclear functions. *International Journal of Molecular Sciences* **17** E1712. (<https://doi.org/10.3390/ijms17101712>)
- Chaiwangyen W, Ospina-Prieto S, Photini SM, Schleussner E, Markert UR & Morales-Prieto DM 2015 Dissimilar microRNA-21 functions and targets in trophoblastic cell lines of different origin. *International Journal of Biochemistry and Cell Biology* **68** 187–196. (<https://doi.org/10.1016/j.biocel.2015.08.018>)
- Chakrabarty A, Tranguch S, Daikoku T, Jensen K, Furneaux H & Dey SK 2007 MicroRNA regulation of cyclooxygenase-2 during embryo implantation. *PNAS* **104** 15144–15149. (<https://doi.org/10.1073/pnas.0705917104>)

- Chan SY, Zhang YY, Hemann C, Mahoney CE, Zweier JL & Loscalzo J 2009 MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. *Cell Metabolism* **10** 273–284. (<https://doi.org/10.1016/j.cmet.2009.08.015>)
- Chang CW, Wakeland AK & Parast MM 2018 Trophoblast lineage specification, differentiation and their regulation by oxygen tension. *Journal of Endocrinology* **236** R43–R56. (<https://doi.org/10.1530/JOE-17-0402>)
- Chaouat G & Clark DA 2015 Are animal models useful or confusing in understanding the human feto-maternal relationship? A debate. *Journal of Reproductive Immunology* **108** 56–64. (<https://doi.org/10.1016/j.jri.2014.10.004>)
- Chen Z, Li Y, Zhang H, Huang P & Luthra R 2010 Hypoxia-regulated microRNA-210 modulates mitochondrial function and decreases ISCU and COX10 expression. *Oncogene* **29** 4362–4368. (<https://doi.org/10.1038/onc.2010.193>)
- Cheong AW, Pang RT, Liu WM, Kottawatta KS, Lee KF & Yeung WS 2014 MicroRNA Let-7a and dicer are important in the activation and implantation of delayed implanting mouse embryos. *Human Reproduction* **29** 750–762. (<https://doi.org/10.1093/humrep/det462>)
- Christie M, Boland A, Huntzinger E, Weichenrieder O & Izaurralde E 2013 Structure of the PAN3 pseudokinase reveals the basis for interactions with the PAN2 deadenylase and the GW182 proteins. *Molecular Cell* **51** 360–373. (<https://doi.org/10.1016/j.molcel.2013.07.011>)
- Colleoni F, Padmanabhan N, Yung HW, Watson ED, Cetin I, Tissot van Patot MC, Burton GJ & Murray AJ 2013 Suppression of mitochondrial electron transport chain function in the hypoxic human placenta: a role for miRNA-210 and protein synthesis inhibition. *PLoS ONE* **8** e55194. (<https://doi.org/10.1371/journal.pone.0055194>)
- Concepcion CP, Bonetti C & Ventura A 2012 The microRNA-17-92 family of microRNA clusters in development and disease. *Cancer Journal* **18** 262–267. (<https://doi.org/10.1097/PP0.0b013e318258b60a>)
- de Rie D, Abugessaisa I, Alam T, Arner E, Arner P, Ashoor H, Astrom G, Babina M, Bertin N, Burroughs AM *et al.* 2017 An integrated expression atlas of miRNAs and their promoters in human and mouse. *Nature Biotechnology* **35** 872–878. (<https://doi.org/10.1038/nbt.3947>)
- Delorme-Axford E, Donker RB, Mouillet JF, Chu TJ, Bayer A, Ouyang YS, Wang TY, Stolz DB, Sarkar SN, Morelli AE *et al.* 2013 Human placental trophoblasts confer viral resistance to recipient cells. *PNAS* **110** 12048–12053. (<https://doi.org/10.1073/pnas.1304718110>)
- Demir R, Seval Y & Huppertz B 2007 Vasculogenesis and angiogenesis in the early human placenta. *Acta Histochemica* **109** 257–265. (<https://doi.org/10.1016/j.acthis.2007.02.008>)
- Denli AM, Tops BB, Plasterk RH, Ketting RF & Hannon GJ 2004 Processing of primary microRNAs by the microprocessor complex. *Nature* **432** 231–235. (<https://doi.org/10.1038/nature03049>)
- Ding J, Huang F, Wu G, Han T, Xu F, Weng D, Wu C, Zhang X, Yao Y & Zhu X 2015 MiR-519d-3p suppresses invasion and migration of trophoblast cells via targeting MMP-2. *PLoS ONE* **10** e0120321. (<https://doi.org/10.1371/journal.pone.0120321>)
- Ding GC, Chen M, Wang YX, Rui C, Xu W, Ding HJ & Shi ZH 2016 MicroRNA-128a-induced apoptosis in HTR-8/SVneo trophoblast cells contributes to pre-eclampsia. *Biomedicine and Pharmacotherapy* **81** 63–70. (<https://doi.org/10.1016/j.biopha.2016.03.040>)
- Donker RB, Mouillet JF, Nelson DM & Sadovsky Y 2007 The expression of Argonaute2 and related microRNA biogenesis proteins in normal and hypoxic trophoblasts. *Molecular Human Reproduction* **13** 273–279. (<https://doi.org/10.1093/molehr/gam006>)
- Doyle M, Badertscher L, Jaskiewicz L, Guttinger S, Jurado S, Hagenschmidt T, Kutay U & Filipowicz W 2013 The double-stranded RNA binding domain of human Dicer functions as a nuclear localization signal. *RNA* **19** 1238–1252. (<https://doi.org/10.1261/rna.039255.113>)
- Ellwanger DC, Buttner FA, Mewes HW & Stumpflen V 2011 The sufficient minimal set of miRNA seed types. *Bioinformatics* **27** 1346–1350. (<https://doi.org/10.1093/bioinformatics/btr149>)
- Escudero CA, Herlitz K, Troncoso F, Acurio J, Aguayo C, Roberts JM, Truong G, Duncombe G, Rice G & Salomon C 2016 Role of extracellular vesicles and microRNAs on dysfunctional angiogenesis during preeclamptic pregnancies. *Frontiers in Physiology* **7** 98. (<https://doi.org/10.3389/fphys.2016.00098>)
- Fabian MR & Sonenberg N 2012 The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. *Nature Structural and Molecular Biology* **19** 586–593. (<https://doi.org/10.1038/nsmb.2296>)
- Farrokhnia F, Aplin JD, Westwood M & Forbes K 2014 MicroRNA regulation of mitogenic signaling networks in the human placenta. *Journal of Biological Chemistry* **289** 30404–30416. (<https://doi.org/10.1074/jbc.M114.587295>)
- Fu G, Brkic J, Hayder H & Peng C 2013a MicroRNAs in human placental development and pregnancy complications. *International Journal of Molecular Sciences* **14** 5519–5544. (<https://doi.org/10.3390/ijms14035519>)
- Fu G, Ye G, Nadeem L, Ji L, Manchanda T, Wang Y, Zhao Y, Qiao J, Wang YL, Lye S *et al.* 2013b MicroRNA-376c impairs transforming growth factor-beta and nodal signaling to promote trophoblast cell proliferation and invasion. *Hypertension* **61** 864–872. (<https://doi.org/10.1161/HYPERTENSIONAHA.111.203489>)
- Fuziwar C & Kimura E 2015 Insights into regulation of the miR-17-92 cluster of miRNAs in cancer. *Frontiers in Medicine* **2** 1–5. (<https://doi.org/10.3389/fmed.2015.00064>)
- Genbacev O, Joslin R, Damsky CH, Polliotti BM & Fisher SJ 1996 Hypoxia alters early gestation human cytotrophoblast differentiation/invasion in vitro and models the placental defects that occur in preeclampsia. *Journal of Clinical Investigation* **97** 540–550. (<https://doi.org/10.1172/JCI118447>)
- Genbacev O, Zhou Y, Ludlow JW & Fisher SJ 1997 Regulation of human placental development by oxygen tension. *Science* **277** 1669–1672. (<https://doi.org/10.1126/science.277.5332.1669>)
- Geng Y, He J, Ding Y, Chen X, Zhou Y, Liu S, Liu X & Wang Y 2014 The differential expression of microRNAs between implantation sites and interimplantation sites in early pregnancy in mice and their potential functions. *Reproductive Sciences* **21** 1296–1306. (<https://doi.org/10.1177/1933719114525273>)
- Grigsby PL 2016 Animal models to study placental development and function throughout normal and dysfunctional human pregnancy. *Seminars in Reproductive Medicine* **34** 11–16. (<https://doi.org/10.1055/s-0035-1570031>)
- Gu Y, Bian Y, Xu X, Wang X, Zuo C, Meng J, Li H, Zhao S, Ning Y, Cao Y *et al.* 2016 Downregulation of miR-29a/b/c in placenta accreta inhibits apoptosis of implantation site intermediate trophoblast cells by targeting MCL1. *Placenta* **48** 13–19. (<https://doi.org/10.1016/j.placenta.2016.09.017>)
- Ha M & Kim VN 2014 Regulation of microRNA biogenesis. *Nature Reviews Molecular Cell Biology* **15** 509–524. (<https://doi.org/10.1038/nrm3838>)
- Hackmon R, Pinnaduwa L, Zhang J, Lye SJ, Geraghty DE & Dunk CE 2017 Definitive class I human leukocyte antigen expression in gestational placenta: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placenta, pregnancy, and parturition. *American Journal of Reproductive Immunology* **77** e12643. (<https://doi.org/10.1111/aji.12643>)
- Han J, Lee Y, Yeom KH, Kim YK, Jin H & Kim VN 2004 The Drosha-DGCR8 complex in primary microRNA processing. *Genes and Development* **18** 3016–3027. (<https://doi.org/10.1101/gad.1262504>)
- Havens MA, Reich AA & Hastings ML 2014 Drosha promotes splicing of a pre-microRNA-like alternative exon. *PLoS Genetics* **10** e1004312. (<https://doi.org/10.1371/journal.pgen.1004312>)
- Hu SJ, Ren G, Liu JL, Zhao ZA, Yu YS, Su RW, Ma XH, Ni H, Lei W & Yang ZM 2008 MicroRNA expression and regulation in mouse uterus during embryo implantation. *Journal of Biological Chemistry* **283** 23473–23484. (<https://doi.org/10.1074/jbc.M800406200>)
- Hu TX, Wang G, Guo XJ, Sun QQ, He P, Gu H, Huang Y, Gao L & Ni X 2016 MiR 20a, -20b and -200c are involved in hydrogen sulfide stimulation of VEGF production in human placental trophoblasts. *Placenta* **39** 101–110. (<https://doi.org/10.1016/j.placenta.2016.01.019>)
- Huntzinger E & Izaurralde E 2011 Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nature Reviews Genetics* **12** 99–110. (<https://doi.org/10.1038/nrg2936>)
- Huppertz B & Peeters LL 2005 Vascular biology in implantation and placenta. *Angiogenesis* **8** 157–167. (<https://doi.org/10.1007/s10456-005-9007-8>)
- Inoue K, Hirose M, Inoue H, Hatanaka Y, Honda A, Hasegawa A, Mochida K & Ogura A 2017 The rodent-specific microRNA cluster within the Sfmbt2 gene is imprinted and essential for placental development. *Cell Reports* **19** 949–956. (<https://doi.org/10.1016/j.celrep.2017.04.018>)
- Ipsaro JJ & Joshua-Tor L 2015 From guide to target: molecular insights into eukaryotic RNA-interference machinery. *Nature Structural and Molecular Biology* **22** 20–28. (<https://doi.org/10.1038/nsmb.2931>)

- Ito M, Sferruzzi-Perri AN, Edwards CA, Adalsteinsson BT, Allen SE, Loo TH, Kitazawa M, Kaneko-Ishino T, Ishino F, Stewart CL *et al.* 2015 A trans-homologue interaction between reciprocally imprinted miR-127 and Rtl1 regulates placenta development. *Development* **142** 2425–2430. (<https://doi.org/10.1242/dev.121996>)
- Ji L, Brkic J, Liu M, Fu G, Peng C & Wang YL 2013 Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. *Molecular Aspects of Medicine* **34** 981–1023. (<https://doi.org/10.1016/j.mam.2012.12.008>)
- Ji L, Zhang L, Li Y, Guo L, Cao N, Bai Z, Song Y, Xu Z, Zhang J, Liu C *et al.* 2017 MiR-136 contributes to pre-eclampsia through its effects on apoptosis and angiogenesis of mesenchymal stem cells. *Placenta* **50** 102–109. (<https://doi.org/10.1016/j.placenta.2017.01.102>)
- Jiang L, Long A, Tan L, Hong M, Wu J, Cai L & Li Q 2017a Elevated microRNA-520g in pre-eclampsia inhibits migration and invasion of trophoblasts. *Placenta* **51** 70–75. (<https://doi.org/10.1016/j.placenta.2017.02.001>)
- Jiang S, Teague AM, Tryggstad JB & Chernausk SD 2017b Role of microRNA-130b in placental PGC-1 α /TFAM mitochondrial biogenesis pathway. *Biochemical and Biophysical Research Communications* **487** 607–612. (<https://doi.org/10.1016/j.bbrc.2017.04.099>)
- Johnson MH & Zimek CA 1981 Induction of polarity in mouse 8-cell blastomeres: specificity, geometry, and stability. *Journal of Cell Biology* **91** 303–308. (<https://doi.org/10.1083/jcb.91.1.303>)
- Jonas S & Izaurralde E 2015 Towards a molecular understanding of microRNA-mediated gene silencing. *Nature Reviews Genetics* **16** 421–433. (<https://doi.org/10.1038/nrg3965>)
- Kang H, Louie J, Weisman A, Sheu-Gruttadauria J, Davis-Dusenbery BN, Lagna G & Hata A 2012 Inhibition of microRNA-302 (miR-302) by bone morphogenetic protein 4 (BMP4) facilitates the BMP signaling pathway. *Journal of Biological Chemistry* **287** 38656–38664. (<https://doi.org/10.1074/jbc.M112.390898>)
- Kang YJ, Lees M, Matthews LC, Kimber SJ, Forbes K & Aplin JD 2015 MiR-145 suppresses embryo-epithelial juxtacrine communication at implantation by modulating maternal IGF1R. *Journal of Cell Science* **128** 804–814. (<https://doi.org/10.1242/jcs.164004>)
- Kaufmann P & Castellucci M 1997 Extravillous trophoblasts in the human placenta – a review. *Trophoblast Research* **10** 21–65. ([https://doi.org/10.1016/S0143-4004\(97\)80079-3](https://doi.org/10.1016/S0143-4004(97)80079-3))
- Kaufmann P, Mayhew TM & Charnock-Jones DS 2004 Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. *Placenta* **25** 114–126. (<https://doi.org/10.1016/j.placenta.2003.10.009>)
- Kawamata T & Tomari Y 2010 Making RISC. *Trends in Biochemical Sciences* **35** 368–376. (<https://doi.org/10.1016/j.tibs.2010.03.009>)
- Kim YK & Kim VN 2007 Processing of intronic microRNAs. *EMBO Journal* **26** 775–783. (<https://doi.org/10.1038/sj.emboj.7601512>)
- Kim YK, Kim B & Kim VN 2016 Re-evaluation of the roles of DROSHA, exportin 5, and DICER in microRNA biogenesis. *PNAS* **113** E1881–E1889. (<https://doi.org/10.1073/pnas.1602532113>)
- Knofler M 2010 Critical growth factors and signalling pathways controlling human trophoblast invasion. *International Journal of Developmental Biology* **54** 269–280. (<https://doi.org/10.1387/ijdb.082769mk>)
- Kolahi KS, Valent AM & Thornburg KL 2017 Cytotrophoblast, not syncytiotrophoblast, dominates glycolysis and oxidative phosphorylation in human term placenta. *Scientific Reports* **7** 42941. (<https://doi.org/10.1038/srep42941>)
- Krawczynski K, Mishima T, Huang X & Sadovsky Y 2016 Intact fetoplacental growth in microRNA-210 deficient mice. *Placenta* **47** 113–115. (<https://doi.org/10.1016/j.placenta.2016.09.007>)
- Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M & Stoffel M 2005 Silencing of microRNAs in vivo with 'antagomirs'. *Nature* **438** 685–689. (<https://doi.org/10.1038/nature04303>)
- Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agostini-Perez FJ, Davuluri R, Liu CG, Croce CM, Negrini M *et al.* 2007 A microRNA signature of hypoxia. *Molecular and Cellular Biology* **27** 1859–1867. (<https://doi.org/10.1128/MCB.01395-06>)
- Kumar P, Luo Y, Tudela C, Alexander JM & Mendelson CR 2013 The c-Myc-regulated microRNA-17–92 (miR-17–92) and miR-106a–363 clusters target hCYP19A1 and hGCM1 to inhibit human trophoblast differentiation. *Molecular and Cellular Biology* **33** 1782–1796. (<https://doi.org/10.1128/MCB.01228-12>)
- Lee RC, Feinbaum RL & Ambros V 1993 The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* **75** 843–854. ([https://doi.org/10.1016/0092-8674\(93\)90529-Y](https://doi.org/10.1016/0092-8674(93)90529-Y))
- Levy R, Smith SD, Chandler K, Sadovsky Y & Nelson M 2000 Apoptosis in human cultured trophoblasts is enhanced by hypoxia and diminished by epidermal growth factor. *American Journal of Physiology: Cell Physiology* **278** C982–C988. (<https://doi.org/10.1152/ajpcell.2000.278.5.C982>)
- Li P, Guo W, Du L, Zhao J, Wang Y, Liu L, Hu Y & Hou Y 2013 microRNA-29b contributes to pre-eclampsia through its effects on apoptosis, invasion and angiogenesis of trophoblast cells. *Clinical Science* **124** 27–40. (<https://doi.org/10.1042/CS20120121>)
- Liang CY, Wang LJ, Chen CP, Chen LF, Chen YH & Chen H 2010 GCM1 regulation of the expression of syncytin 2 and its cognate receptor MFSD2A in human placenta. *Biology of Reproduction* **83** 387–395. (<https://doi.org/10.1095/biolreprod.110.083915>)
- Lipchina I, Elkabetz Y, Hafner M, Sheridan R, Mihailovic A, Tuschl T, Sander C, Studer L & Betel D 2011 Genome-wide identification of microRNA targets in human ES cells reveals a role for miR-302 in modulating BMP response. *Genes and Development* **25** 2173–2186. (<https://doi.org/10.1101/gad.17221311>)
- Lu J, Zhang S, Nakano H, Simmons DG, Wang S, Kong S, Wang Q, Shen L, Tu Z, Wang W *et al.* 2013 A positive feedback loop involving Gcm1 and Fzd5 directs chorionic branching morphogenesis in the placenta. *PLoS Biology* **11** e1001536. (<https://doi.org/10.1371/journal.pbio.1001536>)
- Luo L, Ye G, Nadeem L, Fu G, Yang BB, Honarpour E, Dunk C, Lye S & Peng C 2012 MicroRNA-378a-5p promotes trophoblast cell survival, migration and invasion by targeting Nodal. *Journal of Cell Science* **125** 3124–3132. (<https://doi.org/10.1242/jcs.096412>)
- Luo R, Wang Y, Xu P, Cao G, Zhao Y, Shao X, Li YX, Chang C, Peng C & Wang YL 2016 Hypoxia-inducible miR-210 contributes to preeclampsia via targeting thrombospondin type I domain containing 7A. *Scientific Reports* **6** 19588. (<https://doi.org/10.1038/srep19588>)
- Lyall F, Robson SC & Bulmer JN 2013 Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. *Hypertension* **62** 1046–1054. (<https://doi.org/10.1161/HYPERTENSIONAHA.113.01892>)
- Maltepe E & Fisher SJ 2015 Placenta: the forgotten organ. *Annual Review of Cell and Developmental Biology* **31** 523–552. (<https://doi.org/10.1146/annurev-cellbio-100814-125620>)
- Maltepe E, Bakardjiev AI & Fisher SJ 2010 The placenta: transcriptional, epigenetic, and physiological integration during development. *Journal of Clinical Investigation* **120** 1016–1025. (<https://doi.org/10.1172/JCI41211>)
- Manaster I, Goldman-Wohl D, Greenfield C, Nachmani D, Tsukerman P, Hamani Y, Yagel S & Mandelboim O 2012 MiRNA-mediated control of HLA-G expression and function. *PLoS ONE* **7** e33395. (<https://doi.org/10.1371/journal.pone.0033395>)
- Meijer HA, Kong YW, Lu WT, Wilczynska A, Spriggs RV, Robinson SW, Godfrey JD, Willis AE & Bushell M 2013 Translational repression and eIF4A2 activity are critical for microRNA-mediated gene regulation. *Science* **340** 82–85. (<https://doi.org/10.1126/science.1231197>)
- Miao L, Yao H, Li C, Pu M, Yao X, Yang H, Qi X, Ren J & Wang Y 2016 A dual inhibition: microRNA-552 suppresses both transcription and translation of cytochrome P450 2E1. *Biochimica et Biophysica Acta* **1859** 650–662. (<https://doi.org/10.1016/j.bbarm.2016.02.016>)
- Miller RK, Genbacev O, Turner MA, Aplin JD, Caniggia I & Huppertz B 2005 Human placental explants in culture: approaches and assessments. *Placenta* **26** 439–448. (<https://doi.org/10.1016/j.placenta.2004.10.002>)
- Moffett A & Loke C 2006 Immunology of placentation in eutherian mammals. *Nature Reviews Immunology* **6** 584–594. (<https://doi.org/10.1038/nri1897>)
- Morales-Prieto DM, Ospina-Prieto S, Schmidt A, Chaiwangyen W & Markert UR 2014 Elsevier trophoblast research award lecture: origin, evolution and future of placenta miRNAs. *Placenta* **35** S39–S45. (<https://doi.org/10.1016/j.placenta.2013.11.017>)
- Mori M, Bogdan A, Balassa T, Csabai T & Szekeres-Bartho J 2016 The decidua-the maternal bed embracing the embryo-maintains the pregnancy. *Seminars in Immunopathology* **38** 635–649. (<https://doi.org/10.1007/s00281-016-0574-0>)
- Moser G, Gauster M, Orendi K, Glasner A, Theuerkauf R & Huppertz B 2010 Endoglandular trophoblast, an alternative route of trophoblast

- invasion? Analysis with novel confrontation co-culture models. *Human Reproduction* **25** 1127–1136. (<https://doi.org/10.1093/humrep/deq035>)
- Moser G, Weiss G, Gauster M, Sundl M & Huppertz B 2015 Evidence from the very beginning: endoglandular trophoblasts penetrate and replace uterine glands in situ and in vitro. *Human Reproduction* **30** 2747–2757. (<https://doi.org/10.1093/humrep/dev266>)
- Mouillet JF, Chu T, Hubel CA, Nelson DM, Parks WT & Sadovsky Y 2010 The levels of hypoxia-regulated microRNAs in plasma of pregnant women with fetal growth restriction. *Placenta* **31** 781–784. (<https://doi.org/10.1016/j.placenta.2010.07.001>)
- Mouillet JF, Chu T & Sadovsky Y 2011 Expression patterns of placental microRNAs. *Birth Defects Research Part A: Clinical and Molecular Teratology* **91** 737–743. (<https://doi.org/10.1002/bdra.20782>)
- Mouillet JF, Ouyang YS, Bayer A, Coyne CB & Sadovsky Y 2014 The role of trophoblastic microRNAs in placental viral infection. *International Journal of Developmental Biology* **58** 281–289. (<https://doi.org/10.1387/ijdb.130349ys>)
- Mouillet JF, Ouyang Y, Coyne CB & Sadovsky Y 2015 MicroRNAs in placental health and disease. *American Journal of Obstetrics and Gynecology* **213** S163–S172. (<https://doi.org/10.1016/j.ajog.2015.05.057>)
- Muralimanoharan S, Maloyan A, Mele J, Guo C, Myatt LG & Myatt L 2012 MIR-210 modulates mitochondrial respiration in placenta with preeclampsia. *Placenta* **33** 816–823. (<https://doi.org/10.1016/j.placenta.2012.07.002>)
- Muralimanoharan S, Maloyan A & Myatt L 2016 Mitochondrial function and glucose metabolism in the placenta with gestational diabetes mellitus: role of miR-143. *Clinical Science* **130** 931–941. (<https://doi.org/10.1042/CS20160076>)
- Murray AJ 2012 Oxygen delivery and fetal-placental growth: beyond a question of supply and demand? *Placenta* **33** (Supplement 2) e16–e22. (<https://doi.org/10.1016/j.placenta.2012.06.006>)
- Nadeem U, Ye G, Salem M & Peng C 2014 MicroRNA-378a-5p targets cyclin G2 to inhibit fusion and differentiation in BeWo cells. *Biology of Reproduction* **91** 76. (<https://doi.org/10.1095/biolreprod.114.119065>)
- Niu ZR, Han T, Sun XL, Luan LX, Gou WL & Zhu XM 2018 MicroRNA-30a-3p is overexpressed in the placentas of patients with preeclampsia and affects trophoblast invasion and apoptosis by its effects on IGF-1. *American Journal of Obstetrics and Gynecology* **218** 249 e241.e12–e249.e12 (<https://doi.org/10.1016/j.ajog.2017.11.568>)
- Noris M, Perico N & Remuzzi G 2005 Mechanisms of disease: preeclampsia. *Nature Clinical Practice Nephrology* **1** 98–114; quiz 120. (<https://doi.org/10.1038/ncpneph0035>)
- Nosi U, Lanner F, Huang T & Cox B 2017 Overexpression of trophoblast stem cell-enriched microRNAs promotes trophoblast fate in embryonic stem cells. *Cell Reports* **19** 1101–1109. (<https://doi.org/10.1016/j.celrep.2017.04.040>)
- Ouyang Y, Mouillet JF, Coyne CB & Sadovsky Y 2014 Review: placenta-specific microRNAs in exosomes good things come in nano-packages. *Placenta* **35** S69–S73. (<https://doi.org/10.1016/j.placenta.2013.11.002>)
- Paikari A, C DB, Saw D & Blelloch R 2017 The eutheria-specific miR-290 cluster modulates placental growth and maternal-fetal transport. *Development* **144** 3731–3743. (<https://doi.org/10.1242/dev.151654>)
- Pan Q, Niu H, Cheng L, Li X, Zhang Q & Ning Y 2017 Invasion of trophoblast cell lines is inhibited by miR-93 via MMP-2. *Placenta* **53** 48–53. (<https://doi.org/10.1016/j.placenta.2017.03.008>)
- Papandreou I, Cairns RA, Fontana L, Lim AL & Denko NC 2006 HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metabolism* **3** 187–197. (<https://doi.org/10.1016/j.cmet.2006.01.012>)
- Pringle KG, Kind KL, Sferruzzi-Perri AN, Thompson JG & Roberts CT 2010 Beyond oxygen: complex regulation and activity of hypoxia inducible factors in pregnancy. *Human Reproduction Update* **16** 415–431. (<https://doi.org/10.1093/humupd/dmp046>)
- Rajagopalan S, Bryceson YT, Kuppusamy SP, Geraghty DE, van der Meer A, Joosten I & Long EO 2006 Activation of NK cells by an endocytosed receptor for soluble HLA-G. *PLoS Biology* **4** e9. (<https://doi.org/10.1371/journal.pbio.0040009>)
- Ratsep MT, Felker AM, Kay VR, Tolusso L, Hofmann AP & Croy BA 2015 Uterine natural killer cells: supervisors of vasculature construction in early decidua basalis. *Reproduction* **149** R91–R102. (<https://doi.org/10.1530/REP-14-0271>)
- Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M & Fisher SJ 2004 Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. *Journal of Clinical Investigation* **114** 744–754. (<https://doi.org/10.1172/JCI200422991>)
- Regnault TR, Galan HL, Parker TA & Anthony RV 2002 Placental development in normal and compromised pregnancies – a review. *Placenta* **23** (Supplement A) S119–S129. (<https://doi.org/10.1053/plac.2002.0792>)
- Revel A, Achache H, Stevens J, Smith Y & Reich R 2011 MicroRNAs are associated with human embryo implantation defects. *Human Reproduction* **26** 2830–2840. (<https://doi.org/10.1093/humrep/der255>)
- Reynolds LP & Redmer DA 2001 Angiogenesis in the placenta. *Biology of Reproduction* **64** 1033–1040. (<https://doi.org/10.1095/biolreprod64.4.1033>)
- Robertson SA & Moldenhauer LM 2014 Immunological determinants of implantation success. *International Journal of Developmental Biology* **58** 205–217. (<https://doi.org/10.1387/ijdb.140096sr>)
- Robertson SA, Zhang B, Chan H, Sharkey DJ, Barry SC, Fullston T & Schjenken JE 2017 MicroRNA regulation of immune events at conception. *Molecular Reproduction and Development* **84** 914–925. (<https://doi.org/10.1002/mrd.22823>)
- Rossant J & Cross JC 2001 Placental development: lessons from mouse mutants. *Nature Reviews Genetics* **2** 538–548. (<https://doi.org/10.1038/35080570>)
- Ruby JG, Jan CH & Bartel DP 2007 Intronic microRNA precursors that bypass Drosha processing. *Nature* **448** 83–86. (<https://doi.org/10.1038/nature05983>)
- Schjenken JE, Zhang B, Chan HY, Sharkey DJ, Fullston T & Robertson SA 2016 miRNA regulation of immune tolerance in early pregnancy. *American Journal of Reproductive Immunology* **75** 272–280. (<https://doi.org/10.1111/aji.12490>)
- Schmidt A, Morales-Prieto DM, Pastuszek J, Frohlich K & Markert UR 2015 Only humans have human placentas: molecular differences between mice and humans. *Journal of Reproductive Immunology* **108** 65–71. (<https://doi.org/10.1016/j.jri.2015.03.001>)
- Seitz H, Royo H, Bortolin ML, Lin SP, Ferguson-Smith AC & Cavaillie J 2004 A large imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. *Genome Research* **14** 1741–1748. (<https://doi.org/10.1101/gr.2743304>)
- Su MT, Tsai PY, Tsai HL, Chen YC & Kuo PL 2017 miR-346 and miR-582-3p-regulated EG-VEGF expression and trophoblast invasion via matrix metalloproteinases 2 and 9. *Biofactors* **43** 210–219. (<https://doi.org/10.1002/biof.1325>)
- Sun M, Chen H, Liu J, Tong C & Meng T 2015 MicroRNA-34a inhibits human trophoblast cell invasion by targeting MYC. *BMC Cell Biology* **16** 21. (<https://doi.org/10.1186/s12860-015-0068-2>)
- Szekeres-Bartho J 2002 Immunological relationship between the mother and the fetus. *International Reviews of Immunology* **21** 471–495. (<https://doi.org/10.1080/08830180215017>)
- Takahashi H, Ohkuchi A, Kuwata T, Usui R, Baba Y, Suzuki H, Chaw Kyi TT, Matsubara S, Saito S & Takizawa T 2017 Endogenous and exogenous miR-520c-3p modulates CD44-mediated extravillous trophoblast invasion. *Placenta* **50** 25–31. (<https://doi.org/10.1016/j.placenta.2016.12.016>)
- Truesdell SS, Mortensen RD, Seo M, Schroeder JC, Lee JH, LeTouqueze O & Vasudevan S 2012 MicroRNA-mediated mRNA translation activation in quiescent cells and oocytes involves recruitment of a nuclear microRNP. *Scientific Reports* **2** 842. (<https://doi.org/10.1038/srep00842>)
- Valinezhad Orang A, Safaralizadeh R & Kazemzadeh-Bavili M 2014 Mechanisms of miRNA-mediated gene regulation from common downregulation to mRNA-specific upregulation. *International Journal of Genomics* **2014** 970607. (<https://doi.org/10.1155/2014/970607>)
- Vasudevan S 2012 Posttranscriptional upregulation by microRNAs. *Wiley Interdisciplinary Reviews: RNA* **3** 311–330. (<https://doi.org/10.1002/wrna.121>)
- Viswanathan SR, Mermel CH, Lu J, Lu CW, Golub TR & Daley GQ 2009 microRNA expression during trophobectoderm specification. *PLoS ONE* **4** e6143. (<https://doi.org/10.1371/journal.pone.0006143>)
- Wakeland AK, Soncin F, Moretto-Zita M, Chang CW, Horii M, Pizzo D, Nelson KK, Laurent LC & Parast MM 2017 Hypoxia directs human extravillous trophoblast differentiation in a hypoxia-inducible factor-

- dependent manner. *American Journal of Pathology* **187** 767–780. (<https://doi.org/10.1016/j.ajpath.2016.11.018>)
- Wang Y & Zhao S 2010 Integrated systems physiology: from molecules to function to disease. In *Vascular Biology of the Placenta*. San Rafael (CA): Morgan & Claypool Life Sciences.
- Wang Y, Zhang Y, Wang H, Wang J, Zhang Y, Wang Y, Pan Z & Luo S 2014 Aberrantly up-regulated miR-20a in pre-eclampsic placenta compromised the proliferative and invasive behaviors of trophoblast cells by targeting forkhead box protein A1. *International Journal of Biological Sciences* **10** 973–982. (<https://doi.org/10.7150/ijbs.9088>)
- Wang L, Wang HP, Wang Y & Wang J 2017 MicroRNA-195 is downregulated in the peripheral blood of pregnant women with pregnancy-induced hypertension and inhibits the trophoblast apoptosis through targeting iNOS. *International Journal of Clinical and Experimental Medicine* **10** 716–723.
- Wildman DE, Chen CY, Erez O, Grossman LI, Goodman M & Romero R 2006 Evolution of the mammalian placenta revealed by phylogenetic analysis. *PNAS* **103** 3203–3208. (<https://doi.org/10.1073/pnas.0511344103>)
- Wooding FBP & Burton G 2008 *Comparative Placentation: Structures, Functions and Evolution*. Berlin: Springer.
- Wu Z, Zhang W, Chen G, Cheng L, Liao J, Jia N, Gao Y, Dai H, Yuan J, Cheng L *et al.* 2008 Combinatorial signals of activin/nodal and bone morphogenic protein regulate the early lineage segregation of human embryonic stem cells. *Journal of Biological Chemistry* **283** 24991–25002. (<https://doi.org/10.1074/jbc.M803893200>)
- Wu H, Wang H, Liu M, Bai Y, Li YX, Ji L, Peng C, Yu Y & Wang YL 2016 MiR-195 participates in the placental disorder of preeclampsia via targeting activin receptor type-2B in trophoblastic cells. *Journal of Hypertension* **34** 1371–1379. (<https://doi.org/10.1097/HJH.0000000000000948>)
- Xiao M, Li J, Li W, Wang Y, Wu F, Xi Y, Zhang L, Ding C, Luo H, Li Y *et al.* 2016 MicroRNAs activate gene transcription epigenetically as an enhancer trigger. *RNA Biology* **14** 1326–1334. (<https://doi.org/10.1080/15476286.2015.1112487>)
- Xie M, Li M, Vilborg A, Lee N, Shu MD, Yartseva V, Sestan N & Steitz JA 2013 Mammalian 5'-capped microRNA precursors that generate a single microRNA. *Cell* **155** 1568–1580. (<https://doi.org/10.1016/j.cell.2013.11.027>)
- Xu RH, Chen X, Li DS, Li R, Addicks GC, Glennon C, Zwaka TP & Thomson JA 2002 BMP4 initiates human embryonic stem cell differentiation to trophoblast. *Nature Biotechnology* **20** 1261–1264. (<https://doi.org/10.1038/nbt761>)
- Xu W, San Lucas A, Wang Z & Liu Y 2014 Identifying microRNA targets in different gene regions. *BMC Bioinformatics* **15** (Supplement 7) S4. (<https://doi.org/10.1186/1471-2105-15-S7-S4>)
- Yan T, Liu Y, Cui K, Hu B, Wang F & Zou L 2013 MicroRNA-126 regulates EPCs function: implications for a role of miR-126 in preeclampsia. *Journal of Cellular Biochemistry* **114** 2148–2159. (<https://doi.org/10.1002/jcb.24563>)
- Yang JS & Lai EC 2011 Alternative miRNA biogenesis pathways and the interpretation of core miRNA pathway mutants. *Molecular Cell* **43** 892–903. (<https://doi.org/10.1016/j.molcel.2011.07.024>)
- Yoda M, Kawamata T, Paroo Z, Ye X, Iwasaki S, Liu Q & Tomari Y 2010 ATP-dependent human RISC assembly pathways. *Nature Structural and Molecular Biology* **17** 17–23. (<https://doi.org/10.1038/nsmb.1733>)
- Yu C, Shen K, Lin M, Chen P, Lin C, Chang GD & Chen H 2002 GCMa regulates the syncytin-mediated trophoblastic fusion. *Journal of Biological Chemistry* **277** 50062–50068. (<https://doi.org/10.1074/jbc.M209316200>)
- Yu Y, Wang L, Liu T & Guan H 2015 MicroRNA-204 suppresses trophoblast-like cell invasion by targeting matrix metalloproteinase-9. *Biochemical and Biophysical Research Communications* **463** 285–291. (<https://doi.org/10.1016/j.bbrc.2015.05.052>)
- Zhang H, Kolb FA, Jaskiewicz L, Westhof E & Filipowicz W 2004 Single processing center models for human Dicer and bacterial RNase III. *Cell* **118** 57–68. (<https://doi.org/10.1016/j.cell.2004.06.017>)
- Zhang Y, Fei M, Xue G, Zhou Q, Jia Y, Li L, Xin H & Sun S 2012 Elevated levels of hypoxia-inducible microRNA-210 in pre-eclampsia: new insights into molecular mechanisms for the disease. *Journal of Cellular and Molecular Medicine* **16** 249–259. (<https://doi.org/10.1111/j.1582-4934.2011.01291.x>)
- Zhang J, Dunk C, Croy AB & Lye SJ 2016a To serve and to protect: the role of decidual innate immune cells on human pregnancy. *Cell and Tissue Research* **363** 249–265. (<https://doi.org/10.1007/s00441-015-2315-4>)
- Zhang M, Muralimanoharan S, Wortman AC & Mendelson CR 2016b Primate-specific miR-515 family members inhibit key genes in human trophoblast differentiation and are upregulated in preeclampsia. *PNAS* **113** E7069–E7076. (<https://doi.org/10.1073/pnas.1607849113>)
- Zheng GX, Ravi A, Gould GM, Burge CB & Sharp PA 2011 Genome-wide impact of a recently expanded microRNA cluster in mouse. *PNAS* **108** 15804–15809. (<https://doi.org/10.1073/pnas.1112772108>)
- Zhu X, Yang Y, Han T, Yin G, Gao P, Ni Y, Su X, Liu Y & Yao Y 2015 Suppression of microRNA-18a expression inhibits invasion and promotes apoptosis of human trophoblast cells by targeting the estrogen receptor alpha gene. *Molecular Medicine Reports* **12** 2701–2706. (<https://doi.org/10.3892/mmr.2015.3724>)
- Zhu Y, Lu H, Huo Z, Ma Z, Dang J, Dang W, Pan L, Chen J & Zhong H 2016 MicroRNA-16 inhibits feto-maternal angiogenesis and causes recurrent spontaneous abortion by targeting vascular endothelial growth factor. *Scientific Reports* **6** 35536. (<https://doi.org/10.1038/srep35536>)
- Zou Y, Jiang Z, Yu X, Zhang Y, Sun M, Wang W, Ge Z, De W & Sun L 2014 MiR-101 regulates apoptosis of trophoblast HTR-8/SVneo cells by targeting endoplasmic reticulum (ER) protein 44 during preeclampsia. *Journal of Human Hypertension* **28** 610–616. (<https://doi.org/10.1038/jhh.2014.35>)

Received 3 October 2017

First decision 6 November 2017

Revised manuscript received 12 March 2018

Accepted 3 April 2018