REVIEW

The role of Sirtuin1-PPAR γ axis in placental development and function

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Abstract

Placental development is important for proper *in utero* growth and development of the fetus, as well as maternal well-being during pregnancy. Abnormal differentiation of placental epithelial cells, called trophoblast, is at the root of multiple pregnancy complications, including miscarriage, the maternal hypertensive disorder preeclampsia and intrauterine growth restriction. The ligand-activated nuclear receptor, PPAR γ , and nutrient sensor, Sirtuin-1, both play a role in numerous pathways important to cell survival and differentiation, metabolism and inflammation. However, each has also been identified as a key player in trophoblast differentiation and placental development. This review details these studies, and also describes how various stressors, including hypoxia and inflammation, alter the expression or activity of PPAR γ and Sirtuin-1, thereby contributing to placenta-based pregnancy complications.

Key Words

- ▶ Sirt1
- ▶ PPAR γ
- ▶ placenta
- ▶ trophoblast

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Introduction

Placental development

The placenta is a unique organ present only during intrauterine life, but contributing significantly to fetal growth and development (Burton & Jauniaux 2015). It comprises epithelial cells derived from the trophoblast (TE), the outer layer of blastocyst and of extraembryonic mesodermal (ExM) cells derived from the inner cell mass (ICM), the cells which give rise to the embryo-proper (James *et al.* 2012). Over time, this combination of cells gives rise to an intricate organ, one that not only anchors the fetus within the uterine cavity, but also functions to provide the needed oxygen, nutrients and hormones for fetal growth and expel carbon dioxide and other waste. While the ExM gives rise to the mesenchymal portions of the placenta, including the fetal vasculature, the TE differentiates into two major trophoblast subtypes:

villous trophoblast (labyrinthine trophoblast in mouse), involved in gas and nutrient exchange, and extravillous trophoblast (junctional zone in mouse), which anchor the placenta into the uterine wall and remodel maternal spiral arterioles in order to provide blood flow to the feto-placental unit (Fig. 1) (reviewed in Soncin *et al.* 2015). Abnormal development and/or function of this organ has significant consequences for both mother and baby, leading to complications ranging from gestational hypertension/preeclampsia and fetal growth restriction, gestational diabetes and macrosomia, to preterm delivery and stillbirth (Fisher 2015). In addition, more recent studies have shown that pregnancy complications, particularly those leading to aberrations in fetal growth, have a long-term effect, contributing to metabolic programming of the offspring and thus increasing the risk of obesity, diabetes and cardiovascular disease, later in life (Thornburg &

Marshall 2015). This knowledge necessitates a deeper understanding of placental development, particularly pathways that can affect fetal growth. This review focuses on signaling pathways downstream of the nuclear receptor, PPAR γ and the protein deacetylase, Sirt1, and how these pathways, individually and in combination, affect both the development and the function of the placenta.

PPAR γ and Sirtuin-1

Peroxisome proliferator-activated receptor- γ (PPAR γ) is a member of the family of ligand-activated nuclear hormone receptors, and a transcription factor known for its key role in glucose and lipid metabolism and adipocyte differentiation. Following dimerization with retinoid-X receptors (RXRs), PPAR γ binds to specific DNA sequences termed PPAR γ -responsive elements (PPREs) and induces genes involved in fatty acid uptake and storage, leading to lipid accumulation and adipogenesis. In fact, PPAR γ is required for the formation of both white and brown adipose tissues, the former a site of energy storage and an endocrine organ, and the latter a site of energy expenditure and heat generation. PPAR γ can be targeted using thiazolidinediones (TZDs), a family of synthetic agonists, which are most commonly used in treatment of type 2 diabetes (reviewed in Park *et al.* 2008, Koppen & Kalkhoven 2010).

Sirtuin-1 (Sirt1) is a member of the NAD⁺-dependent family of protein deacetylases and a nutrient sensor. Originally discovered in the budding yeast, *Saccharomyces cerevisiae*, as a gene able to regulate longevity, Sirt1 was first identified as a histone deacetylase, promoting chromatin compaction and hence silencing the genome in times of nutrient deprivation (reviewed in Giblin *et al.* 2014). Since then, studies in mammalian systems have identified numerous non-histone targets of this deacetylase, including p53, FOXO1 and PPAR γ . Notably, Sirt1 induction leads to PPAR γ repression, thus inhibiting adipogenesis and enhancing fat mobilization, with the reverse phenotype shown by RNAi-induced Sirt1 downregulation (Picard *et al.* 2004). In addition to metabolism, Sirt1 has been found to influence numerous other pathways, including those involved in cell proliferation, apoptosis, autophagy and inflammation (reviewed in Knight & Milner 2012, Simmons *et al.* 2015). Sirt1 can be targeted by the naturally occurring compound, resveratrol, which has been identified as an anti-inflammatory agent and anti-oxidant, as well as by synthetic small molecules (Farghali *et al.* 2013).

While the negative regulation of PPAR γ by Sirt1 has been the most well studied, the relationship between these two proteins is by no means simple. A recent study has shown that Sirt1-mediated deacetylation of PPAR γ leads to the recruitment of a co-activator, Prdm1, and selective activation of PPAR γ to promote 'browning' of white fat (Qiang *et al.* 2012). In addition, PPAR γ can also be an upstream negative regulator of Sirt1, both by binding and inhibiting its deacetylase activity and by reducing its transcription (Han *et al.* 2010). Finally, both the TZD class of PPAR γ agonists and the Sirt1 inducer resveratrol have off-target effects, with TZDs inducing transient Sirt1 overexpression (Wei *et al.* 2010), and resveratrol identified to also bind nuclear receptors in the PPAR family, including PPAR γ (Calleri *et al.* 2014). Thus, evaluation of cross-talk between pathways regulated by these two proteins warrants careful experimentation and interpretation of results, particularly when using the common agonists.

In this review, we will focus on the role of the Sirt1-PPAR γ axis in the placenta, with additional focus on trophoblast, the epithelial compartment of the placenta. We will begin by detailing the role of each protein during placental development, and to the extent known, in trophoblast differentiation. Next, we will discuss how these pathways are modulated in placental disease, particularly those associated with the stressors, hypoxia, oxidative stress, inflammation and hyperglycemia. Throughout, we will also address the cross-talk between these two pathways (when known), as well as the therapeutic potential of targeting these pathways in placenta-based complications of pregnancy.

PPAR γ in trophoblast differentiation and placental development

The gene for this protein is located on mouse chromosome 6 and human chromosome 3; in both species, alternative splicing gives rise to multiple isoforms, but the most ubiquitous isoform and the one abundantly present in the placenta as well as in all trophoblast subtypes is PPAR γ 1 (Barak *et al.* 1999). Knockout of this gene in mice first revealed its importance in placental development: in its absence, embryos did not survive past midgestation (Barak *et al.* 1999, Kubota *et al.* 1999), and embryonic lethality could be rescued by either wild-type tetraploid aggregation (Barak *et al.* 1999) or by generation of an epiblast-specific knockout, using Sox2-Cre mice (Duan *et al.* 2007), indicating that placental defects are the basis for the embryonic phenotype. In fact, PPAR γ was expressed

in the placenta, beginning at embryonic day E8.0, prior to its expression in the embryo-proper (at E14.5) (Barak *et al.* 1999). Evaluation of PPAR γ -null placentae showed defects in labyrinth formation, with a thickened chorion persisting at E10.0, just after chorio-allantoic fusion should have taken place (Barak *et al.* 1999). To dissect out the role of PPAR γ in the trophoblast compartment, we derived trophoblast stem cells (TSC) from littermate wild-type (WT) and PPAR γ -null blastocyst-stage (E3.5) mouse embryos (Parast *et al.* 2009). PPAR γ -null TSCs grew slowly and showed altered differentiation *in vitro*, with a complete lack of labyrinthine trophoblast markers, instead showing significantly elevated markers of junctional zone (spongiotrophoblast and trophoblast giant cells/TGCs); in fact, functionally, differentiated PPAR γ -null TSCs invaded Matrigel in greater numbers than differentiated WT-TSCs (Parast *et al.* 2009). Reintroduction of the gene into PPAR γ -null TSCs partially restored the differentiation phenotype, inducing the formation of syncytiotrophoblast and decreasing the number of cells able to invade Matrigel (Fig. 1A) (Parast *et al.* 2009).

The important role of PPAR γ in trophoblast differentiation and placental development is also highlighted by studies using agonists. *In vitro*, treatment of mTSC with rosiglitazone shifted differentiation toward the labyrinthine lineage, and inhibited trophoblast invasion; these effects were absent in PPAR γ -null TSCs, indicating they were PPAR γ dependent (Parast *et al.* 2009). Similar findings have been noted in human trophoblast, both primary extravillous trophoblast and the HPEC65 cell line, with agonists inhibiting invasion in a concentration-dependent manner (Fournier *et al.* 2007). Interestingly, synthetic (troglitazone, a TZD), but not naturally occurring PPAR γ agonists (15deltaPGJ2), enhanced differentiation of primary term cytotrophoblast (CTB) into syncytiotrophoblast (STB), both as assessed morphologically and by hCG secretion (Schaiff *et al.* 2000). Another TZD, rosiglitazone, was found to affect the secretory function of human trophoblast differentially, enhancing transcription of hCG alpha and beta subunits and hCG secretion in villous trophoblast but inhibiting the same in extravillous trophoblast (Handschuh *et al.* 2009). Overall, these data suggest that the effects of PPAR γ activation on trophoblast differentiation are similar between mouse and human, with enhancement of villous/labyrinthine trophoblast and inhibition of extravillous/junctional zone trophoblast, differentiation and function (Fig. 1A). The effects of PPAR γ activation on placental development *in vivo* have also been evaluated, albeit to a lesser extent: continuous treatment with rosiglitazone between E10.5 and E18.5 led to a

Figure 1

Cartoon of trophoblast differentiation, indicating the role of PPAR γ (A) or Sirt1 (B) expression and activation in the process. Terminology in parentheses refers to the mouse. Rosi, Rosiglitazone; Resv, Resveratrol. Arrow with circle at the end indicates an inhibitory effect on the indicated pathway.

reduction in both the thickness of the spongiotrophoblast layer and in labyrinthine vasculature, resulting in smaller WT, but not PPAR γ -heterozygous, embryos and placentas (Schaiff *et al.* 2007). In the same study, rosiglitazone also enhanced fatty acid accumulation in the placenta, but not in the embryo (Schaiff *et al.* 2007).

Limited data are available regarding PPAR γ targets specific to the placenta and trophoblast. Perhaps the most well characterized is the mucin, Muc1, which localizes to the apical membrane of labyrinthine trophoblast, facing the maternal sinusoids (Shalom-Barak *et al.* 2004). Muc1 expression is lost in PPAR γ -null placentas and induced following rosiglitazone treatment of WT mTSC (Shalom-Barak *et al.* 2004). Muc1-null placentas show dilated maternal sinusoids with occasional blood

'lakes,' indicating that its expression is required for proper placental function (Shalom-Barak *et al.* 2004). Our own study pointed to Gcm1, a master regulator of villous/labyrinthine trophoblast differentiation, as a gene downregulated in the absence of PPAR γ (Parast *et al.* 2009). A more recent study has provided evidence for Gcm1 as a potential direct target of PPAR γ in the human choriocarcinoma cell line, BeWo, during induction of syncytialization (Levytska *et al.* 2014). But perhaps the most thorough study of PPAR γ targets in the placenta has been performed by Shalom-Barak *et al.* (2012), in which they used microarray-based RNA profiling to evaluate genes altered in both placentas and TSCs in the absence of PPAR γ . Surprisingly, the vast majority of genes identified in this manner had not been identified as PPAR γ targets in other organs; in addition, a significant proportion (40%) of the differentially expressed genes were similarly altered in RxR α -null placentas, confirming this protein to be a main PPAR γ partner in the placenta (Shalom-Barak *et al.* 2012). Finally, since the bulk of the target genes were functionally linked to metabolism, rather than differentiation or development, the authors come to the thought-provoking conclusion that the severe phenotype of the PPAR γ -null placenta indicates that proper trophoblast differentiation is tightly linked to metabolic function (Shalom-Barak *et al.* 2012).

Sirt1 in trophoblast differentiation and placental development

Similar to PPAR γ , Sirt1 is ubiquitously expressed in both the mouse and human placenta (Chen *et al.* 2006, Lappas *et al.* 2011, (Arul Nambi Rajan *et al.* 2018). However, the phenotype of Sirt1-null embryos is more heterogeneous than PPAR γ -null embryos, ranging from mid-to-late embryonic lethality to early postnatal lethality in inbred strains, depending on the genetic background of the mice (Cheng *et al.* 2003, McBurney *et al.* 2003, Wang *et al.* 2008). Nevertheless, a common phenotype among all strains appeared to be fetal growth restriction. Based on this observation, we obtained Sirt1-heterozygous mice on the Sv129 inbred background from Dr McBurney's group, and set out to evaluate this embryonic phenotype in more detail (McBurney *et al.* 2003). We observed embryonic lethality of Sirt1-null embryos at E13.5, confirmed their fetal growth restriction and observed that the placentas associated with these embryos were also small (Arul Nambi Rajan *et al.* 2018). Further histologic evaluation and *in situ* hybridization for various trophoblast markers revealed abnormalities in both the labyrinth and junctional zone.

We therefore proceeded to derive WT and Sirt1-null TSC, again from littermate E3.5 embryos. In the absence of Sirt1, TSC grew slower, but the primary phenotype was that of blunted differentiation, with reduction of both labyrinthine and junctional zone markers (Arul Nambi Rajan *et al.* 2018) (Fig. 1B). In fact, both *in vitro* and *in vivo*, Sirt1 deficiency led to an accumulation of an Epcam⁺ trophoblast progenitor population; this was accompanied by elevated levels of cMet, a receptor tyrosine kinase, which is required for the maintenance of this labyrinthine progenitor cell population (Ueno *et al.* 2013). It is not immediately clear how Sirt1 deficiency leads to the persistence of cMet or whether this receptor is in fact a direct target of Sirt1's deacetylase activity.

Notably, numerous other signaling pathways, some of which are known to be involved in TSC maintenance and differentiation, were also found to be altered in Sirt1-null TSC (Arul Nambi Rajan *et al.* 2018). Among these were Smad- and Stat-dependent pathways, and also multiple metabolic pathways, including PPAR γ . Interestingly, PPAR γ RNA levels were reduced in differentiated Sirt1-null TSC, as confirmed by qPCR (Arul Nambi Rajan *et al.* 2018); this was somewhat unexpected, as Sirt1 is best known as a negative regulator of PPAR γ in adipose tissue. However, PPAR γ downregulation can also partly explain the blunted differentiation phenotype of Sirt1-null TSC, at least with respect to labyrinthine differentiation. As with PPAR γ , we also evaluated the effect of Sirt1 agonists on TSC differentiation. Treatment with resveratrol during differentiation led to a Sirt1-dependent induction of the labyrinthine marker SynA, though inhibition of the spongiotrophoblast marker Tpbpa appeared to be Sirt1 independent (Fig. 2). This agonist-induced shift towards a trophoblast lineage involved in nutrient exchange is consistent with the role of Sirt1 as a nutrient sensor and implies that Sirt1 may in fact serve a similar function in the placenta as in the embryo proper.

Sirt1 is also expressed in human trophoblast; however, to date, no published studies have addressed the role of this deacetylase in maintenance/differentiation of trophoblast in the human placenta. Similarly, while numerous Sirt1 targets have been identified, affecting pathways as wide-ranging as transcription, metabolism, apoptosis, DNA damage repair and autophagy (Simmons *et al.* 2015), direct targets of Sirt1 in trophoblast and placenta are yet to be explored in detail. Our own study, using microarray-based RNA profiling of WT and Sirt1-null TSCs, before and after differentiation, identified a series of altered genes and pathways, some of which were mentioned earlier (Arul Nambi Rajan *et al.* 2018), and

Figure 2

The effect of resveratrol treatment on mouse trophoblast differentiation. Mouse TSCs were differentiated, as previously described (Arul Nambi Rajan *et al.* 2018), in the presence of 25 μ M resveratrol or DMSO carrier for a total of seven days. qPCR was performed for markers of labyrinthine trophoblast (SynA, A) or junctional zone (Tpbpa, B); values were normalized to 18S RNA and shown as fold change adjusted to the value of wild-type (WT) undifferentiated (day 0) cells. Note that resveratrol induces SynA in a Sirt1-dependent manner (A); however, inhibition of Tpbpa expression is Sirt1-independent, as the basal levels in Sirt1-null cells are further decreased by resveratrol treatment in these cells (B). (A) *Indicates *P* value of 0.0007. (B) *Indicates *P* value of 0.0001 for WT and 0.0057 for Sirt1-null cells.

some of which overlapped with pathways regulated by Sirt1 outside the placenta. However, a detailed proteomic profiling study, particularly one focused on changes in acetylation is needed to identify potential direct targets of placental Sirt1.

Sirt1-PPAR γ signaling in placental disease

Alterations in trophoblast differentiation and placental development are associated with numerous pregnancy complications, including miscarriage, pre-eclampsia, fetal growth restriction and gestational diabetes (Kwak-Kim *et al.* 2014, Fisher 2015, Huynh *et al.* 2015). These

conditions are associated with a suboptimal maternal and placental micro-environment, exhibiting features of hypoxia, oxidative stress, inflammation and/or hyperglycemia. Therefore, we will now attempt to summarize how these changes in micro-environment may affect PPAR γ and/or Sirt1 signaling in the placenta. Since most studies regarding PPAR γ and Sirt1 in trophoblast/placenta have focused on hypoxia, we will begin with this topic.

Hypoxia and PPAR γ

Oxygen tension is an important variable in the placenta, both during normal development and in specific diseases of this organ (reviewed in Chang *et al.* 2018). In conditions of low oxygen tension/hypoxia, numerous pathways are activated which subsequently affect tissue homeostasis. The best studied of these is that of hypoxia-inducible factor (HIF), a complex of two component proteins, the oxygen-stabilized HIF- α and the constitutively expressed HIF- β subunits (Lee *et al.* 2004). The HIF complex is required for placentation, and specifically for differentiation of trophoblast into the invasive lineage (trophoblast giant cells/TGCs in mouse and extravillous trophoblast in human) (Adelman *et al.* 2000, Maltepe *et al.* 2005, Wakeland *et al.* 2017). PPAR γ was known to be affected by hypoxia through the HIF complex: specifically, adipocyte differentiation is inhibited by hypoxia, through a HIF-regulated transcriptional repressor's effect on PPAR γ 2, the isoform specific to adipose tissue (Yun *et al.* 2002). Based on this study, we evaluated the effect of hypoxia on PPAR γ in mouse TSCs. We found that low oxygen tension inhibits PPAR γ expression, but that this effect is independent of HIF (Tache *et al.* 2013). In addition, forced expression of PPAR γ under low oxygen tension partially restored labyrinthine trophoblast differentiation (Tache *et al.* 2013).

The above findings correlate with the hypoxia-associated placental pathology present in the maternal hypertensive disorder of preeclampsia (PE). Abnormal differentiation of syncytiotrophoblast, the lineage analogous to labyrinthine trophoblast in mice, is a hallmark of this disease, and is considered secondary to the reduced maternal blood flow to the placenta due to abnormal spiral artery remodeling by the invasive extravillous trophoblast cells (Fisher 2015). In fact, PE placentas have reduced expression of PPAR γ , as well as reduced levels of GCM1, a major regulator of labyrinthine/syncytiotrophoblast formation and a potential target of

PPAR γ , and its target the fusogenic protein, SYNCYTIN (Chen *et al.* 2004, Langbein *et al.* 2008, He *et al.* 2014). These PE-like syncytiotrophoblast abnormalities have been recapitulated *in vitro* by downregulation of GCM1 in the floating human placental explant model (Baczyk *et al.* 2009). We propose that this phenotype originates with downregulation of PPAR γ under hypoxia, leading to reduced GCM1 and SYNCYTIN levels, which in turn adversely affect syncytiotrophoblast differentiation.

Another hallmark of PE is elevated circulating levels of the anti-angiogenic molecule soluble VEGF receptor-1, also known as soluble Flt-1 (sFlt-1) (Karumanchi & Epstein 2007). While the origin and etiology of elevated sFlt-1 secretion in PE placentas continue to be debated, several studies have shown a link between hypoxia and upregulation of sFlt-1 in human trophoblast (Nagamatsu *et al.* 2004, Li *et al.* 2005, Nevo *et al.* 2006, Munaut *et al.* 2008). Our own study has shown a correlation between syncytiotrophoblast sFlt-1 levels and PE disease severity (Tache *et al.* 2011). PPAR γ activity has been linked to elevated levels of sFlt-1 in a rat model of PE: specifically, pregnant rats treated with a PPAR γ antagonist exhibited PE-like symptoms such as elevated blood pressure, proteinuria and reduced pup weight, and accompanied by increased plasma levels of sFlt-1 (McCarthy *et al.* 2011). Interestingly, a separate study in mice linked reduced *Gcm1* levels to elevated circulating levels of sFlt-1 (Bainbridge *et al.* 2012). Together, these studies indicate that the PPAR γ -*Gcm1* axis may be involved in regulation of sFlt-1. We have also evaluated both sFlt-1 RNA levels and secretion in differentiated mouse TSC, following treatment with the PPAR γ agonist rosiglitazone (Fig. 3). We noted reduced levels of sFlt-1 RNA and secretion following treatment with rosiglitazone; the latter did not affect sFlt-1 levels in WT-TSC subjected to hypoxia or in PPAR γ -null TSC, under either normoxia or hypoxia, indicating that this effect was PPAR γ dependent (Fig. 3).

Finally, PE is also characterized by increased levels of trophoblast apoptosis (Leung *et al.* 2001), a cellular process in which PPAR γ is also involved. Interestingly, when term cytotrophoblast are cultured in hypoxia, differentiation into syncytiotrophoblast is blunted and under severe hypoxia, apoptosis results (Nelson *et al.* 1999, Elchalal *et al.* 2004). Treatment of these cells with the PPAR γ agonist, troglitazone, under hypoxic conditions promoted differentiation and limited the apoptotic damage these cells experienced (Elchalal *et al.* 2004). Together, the data provided here suggest that PPAR γ should be considered as an important therapeutic target in placental diseases such as PE.

Figure 3

The effect of rosiglitazone treatment on sFlt-1 in mouse trophoblast. Differentiated mouse TSCs, either wild-type (WT) or PPAR γ -null, were treated with 1 μ M rosiglitazone or DMSO carrier, either in normoxia (21% oxygen, N) or hypoxia (2% oxygen, H), then subjected to either qPCR (A) or ELISA (B) for sFlt-1. (A) *Indicates *P* value of 0.0005. (B) *Indicates *P* value of 0.0002.

Hypoxia and Sirt1

Compared to the PPAR γ story, the relationship between hypoxia and Sirt1 is more complex. However, several studies have identified Sirt1 as an upstream regulator of HIF- α subunits. Sirt1 can deacetylate HIF-1 α , thus blocking the recruitment of p300 and induction of HIF-regulated genes (Lim *et al.* 2010); conversely, Sirt1 selectively stimulates HIF-2 α activity, thus promoting signaling through this alternate HIF- α subunit during hypoxia (Dioum *et al.* 2009). In turn, Sirt1 gene expression has been found to decrease in hypoxia, in a HIF-dependent manner, thus showing bi-directional signaling between these two pathways (Chen *et al.* 2011). Expression of Sirt1 in trophoblast under hypoxic conditions is yet to be evaluated in detail. Our own studies using mouse TSC have not shown a consistent effect of hypoxia on Sirt1

expression (data not shown). However, one study, using human term primary trophoblast has shown induction of Sirt1 under hypoxia, leading to enhanced expression of the N-myc downregulated gene-1 (NDRG1) and reduced expression of p53, thus promoting cell survival (Chen *et al.* 2006). Nevertheless, more detailed studies are required to determine the relationship between hypoxia, HIF and Sirt1 in both trophoblast and placenta.

Similar to PPAR γ , Sirt1 levels have recently been found to be reduced in syncytiotrophoblast of PE placentas by quantitative immunohistochemistry (Broady *et al.* 2017). The authors hypothesize that, given Sirt1's role in increasing longevity, this finding is likely associated with enhanced cellular senescence noted in PE (Broady *et al.* 2017). Sirt1 activity has also been linked to PE in several studies. In one study, the Sirt1 agonist, resveratrol, was shown to attenuate sFlt-1 secretion, which was induced following treatment of normal human placental explants with either cytokines (including TNF α) or hypoxia (1% oxygen); resveratrol was also able to reduce sFlt-1 secretion from explants from PE placentas, albeit by only 25–30% (Cudmore *et al.* 2012). In a more recent study, resveratrol was used to treat human primary term trophoblast, thereby reducing both secretion and mRNA levels of sFlt-1 (Hannan *et al.* 2017). We recently used differentiated WT and Sirt1-null mouse TSC and showed that treatment with resveratrol did reduce sFlt-1 mRNA and secretion, in a Sirt1-dependent manner; unlike PPAR γ , however, the absence of Sirt1 was associated with significantly lower basal levels of sFlt-1 (Fig. 4). Since PPAR γ levels are decreased in the absence of Sirt1 (Arul Nambi Rajan *et al.* 2018), there are likely one or more other targets through which Sirt1 mediates sFlt-1 basal levels. Finally, resveratrol was also able to reverse the elevated blood pressure and proteinuria in a rat model of PE, induced by treatment with NG-nitro-L-arginine methyl ester (L-NAME) (Zou *et al.* 2014). These data suggest that, while Sirt1 is required for the induction of basal sFlt-1 expression, it may also serve a viable therapeutic target for PE, similar to PPAR γ .

Placental oxidative stress and the effects on PPAR γ and Sirt1

PE placentas have also been associated with oxidative stress (Burton *et al.* 2009), a condition of excess reactive oxygen species (ROS), which can be secondary to hypoxia, ischemia/reoxygenation or decreased levels of antioxidants. Since the effects of hypoxia were discussed earlier, we will focus here on oxidative stress induced by means other than hypoxia. In a rat model of maternal

Figure 4

The effect of resveratrol treatment on sFlt-1 in mouse trophoblast. Differentiated mouse TSCs, either wild-type (WT) or Sirt1-null, were treated with 25 μ M resveratrol or DMSO carrier, then subjected to either qPCR (A) or ELISA (B) for sFlt-1. (A) *Indicates *P* value of 0.0185. (B) *Indicates *P* value of 0.0243.

micronutrient deficiency, diets lacking either folic acid or vitamin B12 during pregnancy led to increased plasma markers of oxidative stress and lower levels of placental PPAR γ mRNA; however, while the placenta weights were not affected, no fetal or maternal consequences were described (Meher *et al.* 2014). With regard to Sirt1, oxidative stress, induced by hypoxanthine/xanthine oxidase treatment of human placental explants, decreased Sirt1 mRNA and protein expression and reduced the expression of the glucose transporter gene, GLUT1, and subsequent glucose uptake (Lappas *et al.* 2012). The latter phenotypes were abrogated by treatment with resveratrol (Lappas *et al.* 2012). Similarly, resveratrol treatment reduced placental oxidative stress and apoptosis in the above-described rat model of L-NAME-induced PE (Zou *et al.* 2014). In our study of mouse TSC, microarray-based RNA profiling identified the glutathione peroxidase genes, Gpx1 and

Gpx3, in the top 10 genes downregulated in Sirt1-null TSCs (Fig. 5A). The Gpx family of proteins helps protect cells against oxidative stress by catalyzing the reduction of organic hydroperoxides and hydrogen peroxide by glutathione (Matsubara *et al.* 2015). Downregulation of Gpx1 and Gpx3 in Sirt1-null TSC, compared to WT-TSC, was confirmed by qPCR and was associated with a higher rate of apoptosis in the Sirt1-null cells (Fig. 5B and C). Whether Sirt1-null TSCs are more susceptible to oxidative stress due to the significant reduction in Gpx enzymes remains to be further evaluated.

Placental inflammation and the effects on PPAR γ and Sirt1

Inflammation in the placenta can be either physiologic or pathologic. An example of the former is the increased inflammation noted in the placenta and fetal membranes

in normal term labor (reviewed in Hadley *et al.* 2017). This pro-inflammatory environment at labor has been associated with no change in PPAR γ (Marvin *et al.* 2000), but a reduction in Sirt1 expression in both fetal membranes (chorion) as well as placental (chorionic villous) tissues (Lappas *et al.* 2011, Kim *et al.* 2013). Human placental Sirt1 levels are sensitive to pro-inflammatory cytokines, and have been shown to decrease following treatment with TNF and IL1 β (Lappas *et al.* 2011). Intriguingly, visfatin/Nampt, an adipocytokine and Sirt1 activator, had a positive correlation with Sirt1 levels and was found to be elevated in placentas of obese women prior to term labor, suggesting a potential mechanism whereby the labor-associated decrease in Sirt1 is prevented, resulting in post-term delivery commonly observed in obese pregnant women (Tsai *et al.* 2015).

Pathologic inflammation in the placenta is associated with PE as well as maternal obesity (Harmon *et al.* 2016, Leon-Garcia *et al.* 2016). Elevated levels of placental inflammation have been associated with reduced placental PPAR γ mRNA expression, in the setting of micronutrient deficiency in pregnant rats (Meher *et al.* 2014). Interestingly, in a mouse model of LPS-induced intrauterine fetal demise (IUID), pre-treatment of pregnant mice with the PPAR γ agonist, rosiglitazone, reduced the rate of IUID from 64% to 16% (Bo *et al.* 2016). This effect was associated with increased nuclear localization of PPAR γ in placental trophoblast, reductions in placental pro-inflammatory factors including IL-6 and TNF α and blockage of LPS-evoked nuclear translocation of NF- κ B in labyrinthine trophoblast (Bo *et al.* 2016). Finally, in a rat model of LPS-induced PE, transplantation of human umbilical cord mesenchymal stem cells (MSCs) led to reduced pro-inflammatory markers, including IL-6 and TNF α , and higher levels of PPAR γ , in the placenta; this was accompanied by lower blood pressure and higher fetal weight, compared to LPS treatment alone (Wang *et al.* 2016).

Less is known about the relationship between obesity-associated placental inflammation and Sirt1/PPAR γ expression. This type of inflammation is characterized by infiltration of T cells and macrophages into chorionic villi, best described as a non-infectious chronic villitis/villitis of unknown etiology; in the setting of maternal obesity, this lesion is twice as common in placentas from female fetuses, a phenomenon that remains unexplained (Leon-Garcia *et al.* 2016). While macrophage infiltration of adipose tissue has been associated with decreased Sirt1 expression (Gillum *et al.* 2011), we have not noted alterations in Sirt1 or PPAR γ expression in placentas

Figure 5

Reduced expression of glutathione peroxidase genes and elevated apoptotic index in Sirt1-null mouse TSCs. (A) Microarray profiling of wild-type (WT) or Sirt1-null TSCs revealed two glutathione peroxidase genes, *Gpx1* and *Gpx3*, to be in the top 10 of genes downregulated in the absence of Sirt1. (B) qPCR confirmed these findings. *Indicates $P=0.0002$ for *Gpx1* and $P=0.0001$ for *Gpx3*. (C) Double-labeling of WT and Sirt1-null TSCs with 7-AAD and annexin V showed doubling of apoptotic index (percent of annexin V⁺/7AAD⁻ cells).

associated with maternal obesity (data not shown). However, in a study using a mouse model of high-fat feeding during pregnancy, we noted reduced Sirt1 and increased PPAR γ expression in placentas (Qiao *et al.* 2015). This was associated with elevated placental lipoprotein lipase (LPL) protein and activity, as well as increased fetal body fat content, suggesting that maternal overnutrition affects fetal development through alterations in the Sirt1-PPAR γ axis (Qiao *et al.* 2015). Whether this phenotype works through inflammatory mediators remains to be seen. We have noted a reduction in Sirt1 levels in WT mouse TSCs treated with IL-6 (data not shown); however, we have not yet evaluated how this is linked to other markers of trophoblast differentiation or function.

Hyperglycemia and placental PPAR γ and Sirt1

Finally, while there have not been any studies on alterations of placental Sirt1 in the setting of maternal diabetes, studies on placental PPAR γ levels in this setting have shown noteworthy results. PPAR γ levels were increased both in human primary trophoblast exposed to hyperglycemic conditions (Cawyer *et al.* 2014), as well as in placentas from streptozotocin-induced diabetic pregnant mice (Suwaki *et al.* 2007). At the same time, multiple studies have identified reduced placental PPAR γ expression, in the setting of gestational diabetes (Jawerbaum *et al.* 2004, Holdsworth-Carson *et al.* 2010, Knabl *et al.* 2014, Capobianco *et al.* 2016), with one of these studies showing decreased levels of this protein in both syncytiotrophoblast and extravillous trophoblast (Knabl *et al.* 2014). Further studies are needed to more thoroughly evaluate the link between maternal diabetes and PPAR γ levels in trophoblast and placenta, with consideration of both maternal glycemic control and associated fetal/neonatal growth outcomes.

Figure 6 summarizes the effects of various stressors on both PPAR γ and Sirt1 levels in the placenta.

Conclusions and future directions

While much remains to be evaluated regarding the function of both PPAR γ and Sirt1, and particularly of the latter, in the placenta, the above studies indicate that both of these proteins play key roles, not just in the development of this important transient organ, but in the various pathologies associated with pregnancy complications. One of the major gaps in knowledge in this area is the role of these two proteins in placental nutrient sensing and the associated effect on fetal growth.

Figure 6

Flow chart indicating relationship between placental PPAR γ (A) or Sirt1 (B) and various stressors. (+) indicates induction and (-) indicates inhibition of expression/activity ('?' indicates insufficient data). *Note that while hyperglycemia has been shown to increase PPAR γ levels, the latter appear to be decreased in the setting of gestational diabetes. See text for further details on this and other stressors affecting either protein.

While the absence of expression of each of these genes is associated with fetal growth restriction, their exact role(s) in placental nutrient sensing remain(s) to be explored. Furthermore, in such follow-up studies, the crosstalk between these two pathways must also be considered. For example, while PPAR γ levels are significantly reduced in the absence of Sirt1 in mouse TSCs, Sirt1 appears to negatively regulate PPAR γ in the placenta, in the setting of maternal high fat feeding, much like its function in white adipose tissue. While these results may be related to differences in the system being probed (single cell type *in vitro* vs the intact tissue *in vivo*), future studies should evaluate changes in expression of one protein, even if the other protein is the sole target. This is most important in experiments with agonists: in this context, TSCs derived from embryos genetically deficient for one or the other protein are highly useful for testing off-target effects. In addition, evaluation of the functions of these proteins should ideally be done alongside accurate measurements of their endogenous activities; this will require development of more optimized activity assays, which can be carried out both *in vitro* and *in vivo*. Finally, further identification and characterization of downstream targets of both placental PPAR γ and Sirt1 are needed, including potential shared targets. Given the availability

of drugs targeting these pathways, future studies have the potential to lead to development of therapeutics for multiple placenta-based pregnancy complications.

Declaration of interest

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

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Author contribution statement

JP performed the experiments that contributed to Figs 4 and 5 and helped with the remaining figures and manuscript revision. K A performed the original literature search for the manuscript, did the experiments that contributed to Figs 2 and 5 of the manuscript and reviewed the final draft. P L contributed to the literature search for the manuscript, did experiments which contributed to data described in the manuscript and reviewed the final draft. M M P wrote the first draft of the manuscript and edited the final version.

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