Mitochondrial – Endoplasmic reticulum interactions in the trophoblast: Stress and senescence

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Abstract
Placental stress has been implicated in the pathophysiology of complications of pregnancy, including growth restriction and pre-eclampsia. Initially, attention focused on oxidative stress, but recently mitochondrial and endoplasmic reticulum stress have been identified. Complex molecular interactions exist among these different forms of stress, making it unlikely that any occurs in isolation. In part, this is due to close physiological connections between the two organelles principally involved, mitochondria and the endoplasmic reticulum (ER), mediated through Ca\(^{2+}\) signalling. Here, we review the involvement of the mitochondria-ER unit in the generation of stress within the trophoblast, and consider consequences for obstetric outcome. Mild stress may induce adaptive responses, including upregulation of antioxidant defences and autophagy, while moderate levels may affect stem cell behaviour and reduce cell proliferation, contributing to the growth-restricted phenotype. High levels of stress can stimulate release of pro-inflammatory cytokines and anti-angiogenic factors, increasing the risk of pre-eclampsia. In addition, chronic stress may promote senescence of the trophoblast, which in other cell types leads to a pro-inflammatory senescence-associated secretory phenotype. Evidence from rodents suggests that a degree of trophoblastic stress develops with increasing gestational age in normal pregnancies. The increase in maternal concentrations of soluble fms-like tyrosine kinase-1 (sFlt-1) and reduction in placental growth factor (PlGF) suggest the same may occur in the human, starting around 30 weeks of pregnancy. Placental malperfusion, or co-existing maternal conditions, such as diabetes, will exacerbate that stress. Amelioration of trophoblastic stress should remain a research priority, but will be difficult due to the complexity of the molecular pathways involved.

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1. Introduction
The syncytiotrophoblast of the human placenta is a highly dynamic tissue, performing a wide range of energy-demanding functions essential for the maintenance of a successful pregnancy and normal growth of the fetus. These functions include the active transport of amino acids, ionic pumping, steroid and peptide hormone synthesis, and secretion of a wide array of growth factors and cytokines. As a consequence, the syncytiotrophoblast is equipped with a large number of mitochondria and quantities of rough endoplasmic reticulum (ER). The demands on these organelles vary depending on the stage of gestation, and in response to maternal and fetal cues. Both mitochondria and the ER must be capable of adaptive responses, and given their roles the signalling pathways involved are central to the maintenance of cellular homeostasis. Indeed, the evidence of considerable cross-talk between the two indicates that they operate as an integrated unit. Here, we review the potential roles of these pathways in trophoblast biology, from differentiation of stem cells to senescence.

2. The mitochondria and endoplasmic reticulum, an integrated unit
Mitochondria are widely recognised as the major source of energy in most eukaryotic cells through their production of ATP. In the syncytiotrophoblast they have additional important functions as the site of synthesis of steroid hormones, as well as being involved in the transport and metabolism of cholesterol [1]. Mitochondria are also known to be highly dynamic organelles, forming a reticular network that undergoes continual fission and fusion, altering their morphology and with it their function [2,3]. During the
differentiation of cytotrophoblast cells prior to fusion with the syncytiotrophoblast, the mitochondria undergo morphological changes that generate smaller, more rounded profiles. Tubulovesicular cristae typical of steroidogenic cells become prevalent in the syncytiotrophoblast later in gestation [4], along with large quantities of ER. The ER is best known as the site of synthesis and post-translational processing of proteins destined for insertion into the cell membrane or for secretion. However, the ER plays additional roles as the major intracellular store of calcium, and the site of loading of peptides onto MHC class I molecules. In addition, lipogenic reactions occur on its outer surface. Perturbations of placental mitochondrial and ER homeostasis therefore have the potential to drive diverse downstream effects.

Because calcium and lipid metabolism are central to the function and regulation of both organelles, there are close physical contacts between their respective membranes for integrative signalling. Mitochondrial and ER membranes come into close apposition at punctate sites somewhat similar to synapses in the nervous system, referred to collectively as the mitochondria-associated ER membrane (MAM). Calcium transporters and ion channels are concentrated at these sites, and flux of calcium between the two organelles is thought to link their functionality in a bidirectional manner [5,6]. Thus, calcium-mediated ER signals may stimulate tricarboxylic acid (TCA) cycle activity, complexes of the mitochondrial electron transport chain and ATP production to meet the demands of protein synthesis. In turn, ER maintenance of intracellular calcium homeostasis through SERCA pumping may be responsive to cellular energy levels, and thus mitochondrial activity. Contact between the two organelles must, however, be closely controlled, since mitochondrial Ca\(^{2+}\) overload can also modulate cell death [6,7]. The ER-mitochondria interface additionally provides a platform for the regulation of a number of other shared functions (see Fig. 2 of Ref. [8]), including autophagy, inflammation, some formation [8], the removal of damaged mitochondria [9], and initiation of the apoptotic machinery. A number of regulatory and chaperone proteins have been identified at MAMs, and so such functions may be responsive to changes in the microenvironment or vulnerable to perturbation by stressors [5,6]. Many of these proteins are also involved in the processes of mitochondrial fission and fusion, suggesting that modulation of mitochondrial morphology in response to stress is a further function of the ER.

In mammalian cells, optic atrophy 1 (Opa1) and the mitofusins (Mfn1 and Mfn2) bring about mitochondrial fusion, whilst dynamin 1-like (Drp1) and fission 1 (Fis1) are involved in the process of fission [10]. Mitochondrial-ER interactions are strongly implicated in playing a key role in mitochondrial fission [8], with ER tubules mediating the formation of mitochondrial constriction sites via actin polymerisation induced by the ER-associated protein invertin 2 (INF2) [11] (see Fig. 1 in Ref. [12]). Actin polymerisation is also proposed to enhance assembly of Drp1 complexes at the outer mitochondrial membrane, bringing about a secondary mechanism of constriction and ultimately mitochondrial fission [11]. The involvement of the ER in fission might therefore explain the changes in mitochondrial morphology that accompany an increase in ER mass during the differentiation of cytotrophoblast cells, thereby enhancing capacity in energy production.

Equally, there is increasing recognition of an interaction between the mediators of mitochondrial fusion and the ER. It has been known for some time that Mfn2 mediates an interaction between the mitochondrial reticulum and the ER [13]. Recently, ablation of Mfn2 in mouse embryonic fibroblasts (MEFs) was found to result in increased connections between the ER and mitochondria, and an increased susceptibility to Ca\(^{2+}\)-overload dependent cell death [14]. It has therefore been proposed that Mfn2 acts as a tethering antagonist, preventing an excessive proximity between the mitochondria and the rough ER [14], whilst a similar function has been proposed for Mfn1 in regulating contacts between the mitochondria and smooth ER [15]. Both Mfn1 and Mfn2 have been shown to play vital roles in embryonic development, underlining the importance of continual mitochondrial fission. However, Mfn2 mutant embryos also show specific and severe disruption of the placental trophoblast giant cell layer [16], which may be due to the high metabolic activity of these cells associated with polyploidy. Loss of function of this trophoblast lineage is thought to contribute to the high incidence of mid-gestation embryonic death in these mutants.

3. Oxidative, mitochondrial and ER stress

Attention has focussed on mitochondria within the syncytiotrophoblast, and more recently the ER, due to their involvement in placental stress. It has been recognised for some time that placental oxidative stress is associated with complications of pregnancy, such as pre-eclampsia and growth restriction [17–19], and it is thought to play a significant role in their pathophysiology.

There are many potential sources of reactive oxygen species (ROS) within the syncytiotrophoblast, such as the detoxification of drugs and xenobiotics by cytochrome P450, the response of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase to growth factors and cytokines, and various other oxidoreductase and cyclooxygenase enzymes. However, the mitochondria are considered to be a major source due to the generation of superoxide radicals through the transfer of electrons onto molecular oxygen by complexes I and III of the electron transport chain [20]. This transfer is particularly significant under hypoxic conditions [21,22]. As the superoxide anion is polarised, it remains within the mitochondrial matrix where it is enzymatically converted to hydrogen peroxide by manganese superoxide dismutase. However, excessive generation of ROS, or comprise of the defences through deficiency of catalytic micronutrients, such as selenium, can result in damage to biological molecules within the matrix. Unlike nuclear DNA, mtDNA is not protected by histones and so is vulnerable to oxidative-induced mutation. Furthermore, proteins encoded by mtDNA are synthesised within the matrix and the protein-folding environment can be disturbed by excessive ROS. Mutations and/or aberrant mitochondrial protein folding may compromise assembly of the subunits of ETC into functional complexes, thereby limiting energy production and increasing the risk of further ROS production [23]. Thus, placental oxidative stress is increased in patients with diabetes mellitus when oxidative phosphorylation is enhanced [24], and the risk of developing hypertensive disorders in pregnancy is inversely related to the maternal selenium concentration [25,26]. Mitochondria isolated from placentas of severely pre-eclamptic patients show a dramatically altered morphology [27], though the extent to which this disruption is exaggerated by the process of isolation is unclear. ROS are also generated through the shorter electron transport chain within the ER that traffics electrons during the formation of disulphide bonds [28]. Under normal physiological conditions this accounts for ~25% of ROS generation within cells, although this proportion may be higher in those with a high secretory output, such as the syncytiotrophoblast.

Because of the coupling of mitochondrial and ER function through MAMs, oxidative, mitochondrial and ER stress are closely interlinked, and each is unlikely to occur in isolation [29–31]. Thus, ROS induce calcium release from the ER through the inositol-1,4,5-trisphosphate (IP3R) and ryanodine receptors, with the ions being taken up by the mitochondria at MAMs [5] stimulating further ROS production and generating a dangerous feed-forward situation. Moreover, excessive mitochondrial fission, resulting in a fragmented network, is also associated with increased ROS
production [3,32], and this may represent a protective mechanism serving to isolate and subsequently remove damaged mitochondria. Fission can result in mitochondrial depolarisation due to a loss of membrane potential (Δψm), targeting such fragmented mitochondria for mitophagy [33]. A number of possible mechanisms that link increased ROS production with increased fission have been proposed, although notably there have been no reports that the fission/fusion proteins are themselves directly redox-sensitive [34]. Mitochondrial fusion can act as a rescue pathway for fragmented mitochondria, including those that undergo transient depolarisation. However, if mitochondrial depolarisation is sustained (as seen if a mitochondrial uncoupler is administered) fusion is inhibited and mitophagy thus favoured [33]. It has been suggested that upregulation of pro-fusion proteins may therefore offset some of the consequences of oxidative stress [34], with a possible role for the ERK-JNK pathway implicated [35]. Such promotion of the molecular mediators of fusion (e.g. Mfn2) would also be likely to alter the interaction between mitochondria and ER, and this may in turn offer some protection under conditions of ER/oxidative stress.

Within mitochondria, ROS can also induce damage to mtDNA and may culminate in opening of the membrane permeability transition pore, leading to defective functioning of the electron transport chain, loss of Δψm and a collapse in ATP production [36]. Alternatively, an adaptive antioxidant response to mitigate against increased ROS at the cost of impaired mitochondrial efficiency may occur via the upregulation of the mitochondrial uncoupling protein, UCP2. UCP2 expression increases towards term in the human placenta [37], and does so similarly in the rat placenta in association with the expression of other antioxidant enzymes [38]. Mild uncoupling leading to a slight loss of Δψ is believed to be protective against ROS, with UCP2 implicated in cytoprotection in a number of tissues, but controversy still surrounds the question of whether UCP2 exhibits uncoupling activity under physiological conditions, not least because this is technically difficult to establish [39]. Moreover, there is presently no evidence to support an antioxidant role for UCP2 in the human placenta [37].

Defective mitochondrial function may promote accumulation of unfolded and misfolded proteins within the matrix, initiating stress. Similarly, loss of calcium homeostasis within the ER impairs the protein folding machinery, resulting in the accumulation of unfolded or misfolded proteins in the lumen, a condition that constitutes ER stress.

4. The unfolded protein response

Protein synthesis is core to many cellular events, and so its regulation is integral to homeostatic mechanisms [40]. In eukaryotic cells, protein synthesis occurs in the cytosol, endoplasmic reticulum and mitochondria. In each compartment, a conserved group of organelle-specific molecular chaperones facilitate efficient folding of nascent, unfolded polypeptides into their final, distinctive conformation. Stress occurs when accumulated unfolded or misfolded proteins exceed the compartment's folding capacity, posing the risk of catastrophic damage. To cope with this risk, organelle-specific signalling pathways, known as unfolded protein responses (UPRs), have evolved. These pathways aim to restore homeostasis by promoting expression of compartment-specific genes to increase molecular chaperones and folding capacity, as well as genes involved in protein degradation to remove the accumulated toxic misfolded proteins.

The protein-folding environment of the ER is guarded by the UPRER, which comprises three signal transduction pathways [41]. They are the PERK (PRKR-like endoplasmic reticulum kinase), ATF6 (activating transcription factor 6) and IRE-1 (inositol-requiring enzyme-1) pathways (Fig. 1). The sensors are located on the luminal side of the ER membrane and are activated by the accumulation of misfolded proteins. PERK is a kinase with two principal targets. Firstly, it phosphorylates eukaryotic initiation factor 2-α (eIF2-α), providing a rapid block to translation and reducing the burden on the folding machinery. Secondly, it activates the transcription factors NRF2 and ATF4; the former directly and the latter through phosphorylation of eIF2-α. The ATF6 and IRE-1 pathways also lead to activation of transcription factors, ATF6 and XBP-1 respectively (Fig. 1). Together, these four transcription factors co-ordinate expression of an array of genes that enhance antioxidant defences and increase the folding capacity of the ER. Thus, uptake of cystine and its conversion to glutathione is stimulated, along with expression of ER chaperone proteins, haemoxigenase enzymes, lipogenesis and synthesis of more ER cisternae [42–44]. If these responses fail to restore ER and redox homeostasis, activation of apoptotic pathways occurs through expression of the protein CHOP, and direct signalling through caspase 4.

Trophoblast cell lines show a graded response to exogenous inducers of ER stress, such as tunicamycin that blocks N-glycosylation of proteins or thapsigargin that disrupts calcium homeostasis. Low doses cause only increased phosphorylation of eIF2-α, while higher doses increase the chaperone proteins and finally activate the CHOP pathway [45]. Teleologically, apoptosis should only be induced as an action of last resort if homeostasis cannot be restored.

Although the UPR pathways evolved to regulate ER function, they have subsequently been recruited to modulate other cellular activities [40]. ChIP-seq analysis in skeletal muscle has revealed that approximately 40% of XBP-1 targets are unrelated to ER function, including, for example, genes associated with myogenic differentiation [46]. Hence, ER stress may have diverse effects.

Increasing evidence suggests the existence of UPR pathways in mitochondria too, UPRmt, which are particularly active in cells with a high rate of biogenesis, high ROS production, and defective mitochondrial structure [47–49]. Conceptually similar to the UPRER, once mitochondria sense unfolded or misfolded proteins, a signal is transmitted to the nucleus, promoting expression of genes encoding mitochondria-specific molecular chaperones and quality-control proteases in order to re-establish mitochondrial homeostasis [50]. The signalling pathways involved in the UPRmt are not well understood. It has been demonstrated that a quality control protease, ClpP, which is located within the mitochondrial matrix, and the ATP Binding Cassette (ABC) transporter, which resides in the mitochondrial inner membrane, may play key roles. ClpP recognizes and degrades misfolded proteins into short peptides, which in turn are extruded into the intermembrane space by the ABC transporter. Knock-down of the ClpP gene prevents mitochondrial stress-induced expression of mitochondrial molecular chaperone genes [50]. Equally, deletion of the ABC transporter orthologous gene, HAF-1 in C. elegans, attenuates UPRmt activation upon mitochondrial stress, suggesting ClpP-derived peptides may act as signalling components in the UPRmt [51]. The UPRmt activates the bZIP transcription factor ATF5-1 (Activating Transcription Factor associated with Stress, also known as ZC376.7), which translocates to the nucleus to stimulate expression of mitochondrial molecular chaperone genes, including mitochondrial heat shock protein 70 (mtHSP70) and HSP60. The transcriptional regulatory function of ATF5-1 requires interaction with another transcription factor, homeobox transcription factor DVE-1, and with co-factor ubiquitin-like protein UBL-5 [50].

5. Involvement of UPR pathways in placental physiology and development

Activation of the UPR pathways should not necessarily be
interpreted as evidence of pathology, and may be a cell-type and condition-specific response to normal events, such as differentiation or variations in physiology. Thus, the pancreas and placenta display low-grade activation of the UPRER under normal conditions because of their high endocrine and exocrine activity [52,53]. Another example is in the crypts of the intestine, where markers of activation of the UPRER pathways only become apparent as the stem cells differentiate into transit amplifying cells, presumably reflecting a change in the requirements for cell surface and secreted proteins. Administration of thapsigargin to organoid cultures of these stem cells causes loss of stemness in a PERK-eIF2-α-dependent manner, indicating the importance of UPRER pathways for stem cell behaviour [54]. Finally, recent evidence indicates mitochondrial stress signaling induces cellular adaptations that reduce the impact of subsequent exposure to lethal stressors [55].

Although there is considerable overlap in the downstream targets of the three arms of the UPRER, they also have unique functions. Thus, it might be expected that in some circumstances components of the UPRER are activated selectively. This is seen during the differentiation of plasma cells when the synthesis of antibodies is upregulated. There is clearly a need to increase the capacity of the ER as part of this process, but it would be counterproductive to inhibit protein translation at the same time. Hence, plasma cell differentiation is associated with stimulation of the IRE-1 and ATF6 pathways, but no phosphorylation of eIF2-α [56].

Limited data are available for the role of these pathways during development of the placenta. Using a transgenic reporter mouse, Iwawaki et al. demonstrated activation of the IRE-1 pathway during normal development at E14.5 [53]. By contrast, activation in the embryo was minimal. Genetic knock-out of the pathway causes aberrant development of the placenta, particularly in the labyrinth zone where there is abnormal vascularisation associated with reduced levels of VEGF. This is accompanied by reduced proliferation of trophoblast cells, but no increase in apoptosis [53]. Overall, it is apparent that placental changes are responsible for the increase in embryonic deaths observed in the mutant mice.

These findings indicate that UPR signalling pathways are important during placental development. The converse is also true, that elevation of ER and mitochondrial stress can perturb normal formation of the organ. In a mouse model of constitutive ER stress due to a dysfunctional mutation in eIF2-α, there is evidence of premature differentiation of trophoblast stem cells at E9.5 [57]. Embryonic fibroblasts isolated from the mutants proliferate at a rate 50% slower than in controls. Activation of the PERK pathway and accumulation of aberrantly glycosylated proteins are only observed in the endocrine junctional zone [57]. Hence, perturbation of ER function in the murine placenta is context-dependent, and predominantly affects cells with a high secretory output. It is notable that pups homozygous for the mutation die post-natally due to severe defects in pancreatic function [58], suggesting there are parallels between the junctional zone and the pancreas.

Administration of tunicamycin to pregnant dams has also been used to test the effects of prolonged ER stress on placental development [59]. This results in reduced placental weight and disruption of placental morphogenesis, with abnormal interdigitation between the labyrinth and junctional zones. There is also reduced vascularisation of the labyrinth zone, and reduced expression of Slc2a1, the GLUT1 glucose transporter, but increased expression of Slc2a3, the GLUT3 transporter.

There are few data available at present that implicate the UPRmt in placental development. The UPRmt can affect cell cycle proteins, slowing cell proliferation or even inhibiting replication of cells. Cells containing defective mitochondrial structures have altered expression of many cell cycle regulators including p19, a cyclin-dependent kinase inhibitor involved in cell cycle arrest at G1 [60]. There is a reduction in the expected number of Clpp-deficient progenies from heterozygous crosses, indicating increased embryonic loss [61]. It is unclear whether fetal growth restriction occurs in these animals, although Clpp-deficient offspring exhibit smaller body size in adulthood [61].

Hence, experimental evidence suggests that UPR pathways play physiological roles in placental development. Oxidative stress has
also been implicated in placental development, but more in terms of stimulating villous regression than proliferation. Towards the end of the first trimester the villi that initially form over the entire surface of the human chorionic sac regress over the superficial pole, leaving the definitive discoid placenta. Regression is temporally and spatially associated with onset of the maternal circulation, and locally high levels of oxidative stress are thought to suppress villous proliferation and induce apoptosis [62,63]. As this process occurs in all pregnancies it is considered physiological.

6. Involvement of UPR pathways in placental adaptive responses

The boundary between physiological and pathological events is inevitably blurred, with these labels being at opposite ends of a spectrum. A pertinent example is the response to chronic hypobaric hypoxia experienced during pregnancy at high altitude. Placentas from normal pregnancies in women of non-indigenous descent at 3100 m in Colorado show increased phosphorylation of eIF2-α compared to sea-level controls, but no other evidence of activation of the UPRER [64]. Additionally, many subunits of ETC complexes were found to be down-regulated without a change in mitochondrial density, indicating defective mitochondrial function and potential activation of UPRmt [65]. These findings can be replicated by exposing placental cells to hypoxia in culture, and is associated with reduced cell proliferation. Treatment of normoxic cells with salubrinial, a p-eIF2-α phosphatase inhibitor, also suppresses proliferation, and reduces expression of subunits in ETC complexes, confirming that phosphorylation of eIF2-α alone is sufficient to account for the reduced volume of the placental villous trees measured in these placentas [64,66]. A similar increase in phosphorylation of eIF2-α and reduction in mitochondrial ETC subunits occurs in the murine placenta when dams are maintained under 13% oxygen throughout gestation, equivalent to pregnancy at 3100 m [67]. In both cases there is mild growth restriction of the fetus, and so the changes may be viewed as an adaptive response that matches growth to oxygen availability.

Attenuation of protein translation is a part of a cell’s repertoire of adaptive responses to cope with hypoxia [68], for incorporation of a single amino acid into a nascent polypeptide chain requires four high-energy phosphate bonds. The consequences will depend on the duration of the attenuation and the half-life of the proteins concerned, but we have observed further links between ER stress and mitochondrial function through this mechanism. Thus complexes of the mitochondrial electron transport chain are reduced at the protein, but not mRNA, level in the high-altitude placenta, consistent with the reduction in ATP/ADP ratio observed [65,69]. This effect may have been mediated through the PERRK-eIF2-α pathway, for administration of salubrinial to trophoblast-like cells is sufficient to reduce the complexes at the protein level, with a particular stark loss of complex I [65]. While reducing mitochondrial activity might at first sight be considered an adverse response, it is potentially beneficial as long as it is matched by reduced energy demands through translational arrest and/or an increased reliance on glycolysis for ATP production [70–72]. Production of ROS at complexes I and III is paradoxically increased during hypoxia due to an accumulation of electrons on the electron transport chain in the absence of oxygen as the final electron acceptor [21,22,70]. By reducing these complexes, cells may protect themselves from excessive generation of ROS under these conditions. It is notable that a similar reduction in electron transport chain complexes is observed in skeletal muscle of climbers at high altitude [73,74] in association with an altered energetic profile [75].

7. Involvement of UPR pathways in placental pathology

Many common, non-infective complications of pregnancy are associated with deficient physiological conversion of the maternal spiral arteries supplying the placenta [76]. It is frequently asserted that this leads to placental hypoxia, although no measurements have been recorded from the intervillus space in vivo to confirm or refute this supposition. Whether the problem is one of hypoxia or ischaemia-reperfusion-type injury has yet to be resolved [19], but there is conclusive evidence of oxidative stress in placentas from patients suffering fetal growth restriction and pre-eclampsia. Activation of the UPRER is seen in growth-restricted placentas of maternal vascular origin, and in placentas from cases of early-onset, but not late-onset, pre-eclampsia [45,77]. The level of activation is greater, and involves more pathways, than is seen in the normal placentas from high altitude, indicating a more severe insult. In the growth-restricted placentas, levels of AKT were reduced at the protein, but not mRNA, level due to translational arrest, along with reduced cyclin D1. When comparing with gestationally age-matched placentas from pregnancies where the growth restriction was complicated by pre-eclampsia, the levels p-eIF2-α and ER chaperone proteins were even higher, and there was additional activation of the CHOP pro-apoptotic pathway [45].

In comparison, the evidence of UPRmt involvement in pathological pregnancies is very limited. There is an increase of mitochondrial molecular chaperone HSP60 immunoreactivity in the syncytiotrophoblast and cytotrophoblast cells of growth-restricted placentas, especially in regions of thrombi, syncytial knots and avascular villi [78], but no other data.

The changes in the AKT-mTOR pathway, a central regulator of cell proliferation, and in cyclin D1 are sufficient to explain the growth restricted phenotype, but can differences in the placental pathophysiology explain the superimposition of pre-eclampsia on this phenotype? Pre-eclampsia, especially the early-onset form, is an inflammatory state in which elevated serum concentrations of anti-angiogenic and pro-inflammatory cytokines combine to cause maternal endothelial activation. It is generally assumed that the placenta is the source of these factors, as delivery is followed by a rapid decline in their levels, although a contribution from maternal sources cannot be ruled out. Generation of oxidative stress in the placenta, either in vitro through hypoxia-reoxygenation or in vivo through the ischaemia-reperfusion that accompanies labour, mimics the transcriptional changes seen in pre-eclampsia. It also stimulates secretion of pro-inflammatory cytokines and anti-angiogenic factors, including soluble fms-like tyrosine kinase-1, sFlt-1, which has been implicated in the pathophysiology of pre-eclampsia [79–82]. Secretion is mediated through the p38, NfκB, and stress-activated protein kinase MAPK pathways [83].

Again, there are multiple links between the UPRER and these pathways (Fig. 2). Although IRE-1 is generally considered an endoribonuclease that splices XBP-1 mRNA to yield a functional transcription factor, at high levels of activation it has an additional kinase activity and is capable of phosphorylating TRAF2. This in turn activates the p38, NfκB pathways and JNK [84,85]. Furthermore, attenuation of protein translation leads to activation of NfκB, as the half-life of the inhibitory sub-unit, IkB, is shorter than that of NfκB [86]. Hence, it is not unreasonable to envisage that the obstetric outcome is dependent on the severity of the UPRER response, which in turn may reflect interactions between the degree of the vascular insult and maternal factors, such as her genotypic predisposition to endothelial disease and the state of her antioxidant defences. In support of this hypothesis, the few data available indicate that spiral arterial conversion is most deficient in cases of growth restriction associated with pre-eclampsia [87]. Magnetic resonance imaging has also demonstrated reduced perfusion of the
placenta in cases of early-onset but not late-onset pre-eclampsia [88], and a change in the phosphodiester/phosphomonoester ratio of the $^{31}$P signal indicates accelerated ageing of the tissues [89].

8. Syncytiotrophoblast senescence

One of the consequences of chronic oxidative, mitochondrial and ER stress is that they can induce senescent changes in tissues [60,90,91]. Senescence is characterised by irreversible cell-cycle arrest, cytological and metabolic changes and the acquisition of a senescent-associated secretory phenotype (SASP) that leads to the release of a mix of pro-inflammatory cytokines and proteases. Whilst cause and effect can be difficult to establish, senescence is strongly associated with a number of mitochondrial perturbations, which in turn share a connection with ER stress. These include excessive ROS production, increased mitochondrial fusion, mitochondrial uncoupling and depolarisation of the inner mitochondrial membrane, loss of ATP and activation of AMPK, decreased NAD$^{+}$ availability and mitochondrial Ca$^{2+}$ accumulation [10], while pharmacological inhibition of electron transport chain complexes I, II or III can also lead to premature senescence [10,92–94].

Senescence has only recently been considered as a biological phenomenon in the syncytiotrophoblast [95,96], although changes associated with ageing have long been recognised morphologically [97,98]. It has been suggested that the retroviral-driven fusion process by which differentiated cytotrophoblast cells are incorporated into the syncytiotrophoblast initiates the process, and molecular markers of senescence are displayed by the syncytiotrophoblast of mature, but otherwise normal, placentas [95]. Certainly, mitosis has never been reported in the syncytiotrophoblast, and aggregations of nuclei displaying condensed chromatin and evidence of oxidative damage accumulate as gestation advances [99]. Therefore, it may be the case that the syncytiotrophoblast undergoes a degree of molecular senescence in all pregnancies, triggered perhaps by increasing stress arising from a progressive mismatch between maternal perfusion and fetal demands [100] (Fig. 3). This is difficult to prove as longitudinal sampling of the human placenta cannot be performed, but circumstantial evidence from circulating biomarkers indicative of placental well-being suggest that this may be the case. For example, maternal concentrations of cell-free fetal DNA, which can be released by the placenta when subjected to oxidative stress [101], increase steeply after 30 weeks of gestation [102].

![Fig. 2. Summary of the mitochondrial and ER pathways that may contribute to the activation of proinflammatory pathways in the syncytiotrophoblast under conditions of stress.](image)

![Fig. 3. Schematic representation of changes in oxygen concentration in the intervillous space (IVS) of the placenta, fetal weight, and maternal concentrations of sFlt-1 and PlGF across gestational age. A burst of oxidative stress is observed in the trophoblast at the end of the first trimester, associated with onset of the maternal arterial circulation and remodelling of the early placenta into the definitive form. Placental secretion of sFlt-1 is positively regulated by oxidative stress, while that of PlGF is negatively regulated by ER stress. The changes in maternal concentrations may reflect an increase in stress within the trophoblast towards term induced by a progressive mismatch in fetal demand for oxygen and maternal supply. This may be exacerbated in cases of early-onset pre-eclampsia (dashed line) due to malperfusion secondary to deficient remodelling of the spiral arteries. Chronic stress may induce senescent changes in the trophoblast, but the point at which that occurs is uncertain.](image)
Longitudinal studies in rodent models have yielded more solid evidence. An increase in oxidative stress is observed during late gestation in the labyrinth zone that performs gaseous exchange in the mouse [103], and is associated with an increase in the mitochondrial DNA copy number [104]. Similar changes in oxidative stress have been reported in the rat [38]. Increasing stress within the syncytiotrophoblast during the third trimester could explain the gradual increase in maternal serum concentration of sFlt-1 from 29 to 32 weeks onwards [105]. In addition, maternal concentrations of placental growth factor (PIGF) decline after the same time-point. This factor is negatively regulated in trophoblast-like cell lines by the ATF4 and ATF6 pathways [106], and so again the data are consistent with increasing placental stress. Thus, it may be that the changes that occur in complications of pregnancy are an exaggeration of normal placental ageing, induced by malperfusion secondary to deficient remodelling of the spiral arteries (Fig. 3). In support of this hypothesis, shortening of telomeres, a hallmark of senescence, is greater in placentas from pregnancies complicated by growth restriction, with or without pre-eclampsia, but not from those with pre-eclampsia alone [107–109].

Many components of the SAPS, including IL-1, IL-6, IL-8, are increased in early-onset pre-eclampsia and contribute to the maternal inflammatory state. The inflammatory components of the SASP are regulated principally through high-mobility group box 1 (HMGB1) and the NFkB pathway [110], demonstrating the overlap among the oxidative stress, UPR and senescence signalling cascades. HMGB1 signalling is increased in the syncytiotrophoblast in cases of severe pre-eclampsia [111], creating what might be considered a sterile inflammatory response. In normal situations, the function of the SAPS is to attract immune cells that will remove the senescent one. In the case of the syncytiotrophoblast that is not possible due to its unique syncytial nature. Consequently, the response may have been preserved in the cellular machinery, but has the detrimental effects of activating maternal immune and endothelial cells, resulting in the syndrome of pre-eclampsia. Viewing the pathophysiology in this way may open new avenues for therapeutic interventions.

9. Autophagy in the placenta

One of the protective mechanisms cells can deploy against senescence is autophagy, whereby aggregates of misfolded proteins or damaged organelles are degraded through the lysosomal pathway and their constituent elements recycled. There is increasing evidence that prolonged activation of the UPR leads to cell death [112,113]. Additionally, there are also close links between the UPR and immune response, and various components of the autophagic machinery [30,114,115]. It might be expected, therefore, that autophagy occurs in the syncytiotrophoblast in pre-eclamptic or growth restricted pregnancies, but the evidence from different studies is conflicting. Most have relied on changes in the levels of key regulators, such as LC3-II, LAMP-2 and beclin-1, at the mRNA or protein level in placental homogenates as markers. On this basis, increased autophagy has been reported in placentas from pregnancies complicated by growth restriction, with or without pre-eclampsia, but not from those with pre-eclampsia alone [116,117]. Conversely, other studies have found evidence of an increase in placenta from pregnancies complicated by hypertensive disorders, independent of the presence of growth restriction [118,119]. Few studies have included electron micrographs localising the phenomenon, but those available indicate that it occurs in the syncytiotrophoblast and to a lesser extent in cytotrophoblast cells, and involves mitochondria in growth-restricted pregnancies [116–118]. Whilst autophagy can be induced in primary cultures of cytotrophoblast or trophoblast cell lines in vitro through exposure to hypoxia and/or glucose deprivation [120–122], the significance of this response for placental function and fetal well-being in vivo remains uncertain. Nonetheless, the possibility remains that the placenta may act as a nutritional reserve that can be mobilised to protect growth of the fetus, in particular the brain, under conditions of acute deprivation [123].

10. Apoptosis of trophoblast

If homeostatic pathways fail to restore metabolic equilibrium, then oxidative, mitochondrial and ER stress can induce apoptosis in many cell types through activation of both mitochondrial-dependent and –independent apoptotic machineries, including the caspase pathway and CHOP. For the syncytiotrophoblast this presents a major threat, as in the absence of cell boundaries there is a danger of apoptosis sweeping through the whole epithelium, leading to pregnancy failure. Such a wave has been observed in in vivo models of the syncytiotrophoblast [124], but in vivo the syncytiotrophoblast appears resistant to apoptosis, possibly to prevent such a catastrophic event [125]. By contrast, cytotrophoblast cells are vulnerable to both apoptotic and necrotic cell death [125,126], and the incidence is increased in complications of pregnancy, such as miscarriage, pre-eclampsia and growth restriction [127,128], when it may reflect elevated levels of stress. Excessive loss of cytotrophoblast cells through this mechanism may have detrimental consequences for placental function by compromising the capacity for regeneration of the syncytiotrophoblast through fusion. Recruitment of cytotrophoblast cells into the syncytiotrophoblast in the decidua appears to continue through to term [129], and as well as expanding the tissue it brings in fresh mitochondria, ER, and other organelles that may replace aged or damaged examples removed through autophagy. Unfortunately, there are no data available to indicate the rate of such turnover in the syncytiotrophoblast of either healthy or pathological placentas in vivo.

11. Broader implications of ER and oxidative stress for placental pathology

Most attention with regards to oxidative and ER stress has focused on the placenta and the villous trophoblast, but there are reports of increased stress in the decidua in pathological pregnancies. Activation of the PERK-eIF2α and ATF6 pathways has been reported in decidual cells, extravillous trophoblast and macrophages in cases of early-onset growth restriction with and without pre-eclampsia, but not in cases of pre-eclampsia alone [130]. Increased oxidative and ER stress has also been observed in the decidua of cases of early pregnancy loss [131]. In both situations it is difficult to distinguish between cause and effect, but stress responses may impair trophoblast invasion or induce excessive apoptosis, compromising spiral artery remodelling.

In addition, it is possible that ER stress may affect interactions with the maternal immune cells. Extravillous trophoblast cells express the non-polymorphic class I antigens HLA-G and HLA-E, and also the highly polymorphic HLA-C. Interactions between HLA-C and the killer immunoglobulin-like receptors on the uterine natural killer cells are crucial for a successful pregnancy, and indeed appear to regulate birth weight across the natural range [132]. In particular, it is necessary to have a sufficient degree of activation of the natural killer cells, which is thought to mediate their release of proteases and cytokines necessary for remodelling of the maternal arteries. Since peptides are loaded on to MHC molecules within the ER lumen it is possible that loss of ER homeostasis in an extravillous trophoblast cell may compromise its antigenic profile. It is notable that treatment of thyroid cells with thapsigargin or tunicamycin...
reduced MHC class I expression and was associated with increased natural killer cell cytotoxicity [133]. One might speculate, therefore, that induction of ER stress in the invading extravillous trophoblast, possibly induced by low-grade inflammation within the decidua or maternal metabolic disorders, might compromise activation of the uterine natural killer cells, so impairing maternal arterial remodeling with the end result of placental malperfusion and growth restriction and/or pre-eclampsia.

12. Conclusion

Mitochondria and the endoplasmic reticulum are two of the most dynamic and important cell organelles, performing functions central to homeostasis, viability, and growth. Their functional interdependence requires that their activities are closely interlinked, which is achieved through bidirectional signalling at MAMs. This signalling is so extensive they may be considered physiologically as single unit, the function of which may be perturbed by changes in oxygenation, glucose availability and other environmental cues. The extensive complement of both organelles in the syncytiotrophoblast necessary to meet its high metabolic and secretory demands increases with gestational age, and so a degree of stress may be a feature of all otherwise healthy, mature placentas. High levels of trophoblastic stress are associated with complications of pregnancy, and attenuation of protein synthesis in the chorionic villi is part of the Pre-eclampsia-Eclampsia Monitoring, Prevention and Care 21 (10) (1998) 1753–1758.

Conflicts of interest

The authors have no conflicts of interest to declare.

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G.J. Burton et al. / Placenta 52 (2017) 146–155


[107] C.J. Burton et al. / Placenta 52 (2017) 146–155