The Endoplasmic Reticulum Stress of Placental Impoverishment

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If mitochondria are the power packs of the cell, then the endoplasmic reticulum (ER) is the industrial complex, where secretory proteins and other factors are synthesized. Disruption of this organelle, such as by ER stress, can wreak havoc not just on the individual cell but also on the organism as a whole. One such example of this is the ER stress that occurs during the placental stress of intrauterine growth restriction. In this issue of *The American Journal of Pathology*, Yung and colleagues\(^1\) publish a landmark paper in which the role of the ER in placental stress and its associated clinical syndromes is elucidated. To understand fully the importance of the ER in intrauterine growth restriction, one must appreciate the intricacies of ER function.

Nearly all eukaryotic cells contain the ER, a labyrinthine network of interlinked flattened sacs with a continuous cavity that is separated from the cytoplasm by the ER membrane. The ER’s specialized intracellular environment is very high in Ca\(^{2+}\) and oxidizing potential.\(^2\) Ribosomes transiently attached to the outside of the membrane are the defining feature of the rough ER. The smooth ER, on the other hand, is devoid of ribosomes but is rich in enzymes that synthesize lipids and membrane phospholipids and participates in steroid synthesis, in specific cell types. Rough and smooth ER are interconnected, not physically discrete, and the relative proportion of each quickly changes.

As each new peptide is synthesized by the ribosomes of the rough ER, the elongating amino acid chain is extruded into the ER lumen; subsequently whereas the ribosome detaches once its job is done. The naïve peptide enters the ER and folds spontaneously to minimize exposure of hydrophobic areas. Successful protein folding requires a controlled environment of substrates that include glucose to supply energy and calcium and redox buffers that maintain the oxidizing environment required for disulfide bond formation.\(^2\) Further conformational adjustments are achieved and stabilized by N-linked glycosylation and formation of disulfide bonds catalyzed by protein sulfide isomerases. The final product, thus packaged, travels to the Golgi in vesicles that are nipped off from the ER membrane.

Quality control is required for all production lines; in the ER this involves checking for unfolded or misfolded peptides.\(^3\) This involves a series of glycosylation and deglycosylation reactions that enable correctly folded proteins to be distinguished from misfolded ones. The latter may be recycled within the ER for refolding or may be exported, ubiquitinated, and catabolized in lysosomes. ER folding is assisted by ER-specific chaperones\(^4\) that promote noncovalent folding and unfolding of macromolecular structures but are not part of these structures in their normal functional state. They prevent newly synthesized polypeptide chains and assembled subunits aggregated into nonfunctional proteins. For this reason some chaperones are also heat shock proteins because the stress of heat aggregates cell proteins. Others were discovered by the impact of another stressor, glucose starvation, and are called glucose regulated proteins (GRP78, GRP94). New peptides are modified by N-linked glycans. Sequential trimming of sugar residues is monitored by lectins (calnexin and calreticulin), and if protein conformations are satisfactory, these chaperones assist in their export to the Golgi or, if not, target them for ER-assisted degradation.

ER stress occurs when there is an imbalance between the cellular demand for ER function and ER capacity. This is detected by GRP78 (BiP), which is normally anchored to receptors on the luminal aspect of the ER membrane. Excessive misfolded proteins compete to bind GRP78, which, on detachment, releases its membrane receptors to function as alarms that activate the unfolded protein response. This involves an ER-to-nucleus signaling pathway that resets cellular activity to enable normality to be restored. Several protective actions ensue.

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The production line is slowed by reducing peptide synthesis, more help is summoned by increasing production of chaperones to cope with the gridlock of misfolded proteins and to begin their removal, and, if rescue is not achieved, apoptosis is activated. The responses are triggered when three ER stress alarms on the ER membrane are released by GRP78: PERK (protein kinase-like endoplasmic reticulum kinase), ATF6α (activating transcription factor 6α), and Ire1 (inositol-requiring enzyme 1). Activated PERK phosphorylates eukaryotic initiation factor2α (eIF2α), which is the key to the protein production line: phosphorylation attenuates its activity and reduces new protein synthesis and hence the associated burden on the secretory pathway. This gives time to resolve the ER congestion.

Specific transcription factors are activated to redirect cellular activity to the synthesis of proteins that are needed to resolve the stress. Misfolded proteins are partially demannosylated, which marks them for destruction by specialized lectins (ER degradation-enhancing mannosidase-like proteins) that promote the process of ER-assisted degradation. The other two stress sensors (ATF6 and Ire1) cooperate, after activation, in transcriptional induction of UPR target genes (particularly ATF4 and the transcription factor XBP-1), which produce more chaperones and promote ER-assisted degradation of misfolded proteins. IRE1 is a highly conserved, stress-activated endonuclease that participates in an unusual form of mRNA splicing, not in the nucleus but in the cytoplasm (cytoplasmic splicing). The spliced mRNA stimulates production of active XBP-1 protein. ATF4 is a transcription factor that stimulates apoptosis pathways.

Waste management is an essential part of cellular and ER health. Some misfolded proteins can be recycled for refolding within the ER, and those that cannot are degraded by one of two methods. One involves translocation of the protein into the cytosol, ubiquitination, and proteasomal digestion. The other, more applicable to larger more insoluble protein complexes, is autophagy. Autophagy is a catabolic process involving the degradation of a cell’s own components through the lysosomal machinery. At its most extreme, it is a form of cellular cannibalism. Failure of waste disposal, for example by proteasome inhibition, can also stimulate ER stress, providing an example of how cell stress in one part of a cell does not occur in isolation from other parts.

If recovery systems are not adequate, then IRE1 may associate with TRAF2 (tumor necrosis factor receptor-associated factor 2), which is one of a family of TRAF proteins that regulate downstream effects of ligand engagement of tumor necrosis factor receptors. It is a strong activator of ASK1 (apoptosis signal-regulating kinase 1), which is a member of the mitogen-activated protein kinase kinase kinase family. Apoptosis is induced by stimulating the JNK/P38 pathway, especially in the context of prolonged oxidative stress. A mark of apoptosis induced by ER stress is increased synthesis of the transcription protein CHOP, which sensitizes cells to apoptosis by down-regulating the mitochondrial protein BCL2.

The key event of ER stress is stopping new protein synthesis, at the eIF2α phosphorylation switch; however, protein synthesis inhibition occurs after a wider range of cellular stresses. Three other kinases can activate the eIF2α switch, such as in response to amino acid deprivation (GKN2, general control non-essential-2), hemin deficiency (HRI, heme-regulated inhibitor kinase), or viral infection (PKR, activated by double-stranded RNA and interferon-γ).

ER stress can be provoked by many other stimuli or stressors, some of which perturb the specialized milieu of the ER. This comprises an extremely high intra-ER Ca²⁺ concentration, three orders of magnitude more than that of the cytoplasm, and a strong oxidizing environment. Pathophysiological stimuli include heat or osmotic shock, viral infection, hypoxia or oxidative stress, homocysteine, sugar/glucose or amino acid deprivation, UV irradiation, or cholesterol overload. Chemical stimuli used in laboratory experiments include glycosylation inhibitors such as tunicamycin (which prevents control of protein folding), Ca²⁺ metabolism disruptors such as calcium ionophores, or Ca²⁺ pump inhibitors such as thapsigargin. Reducing agents that disrupt disulfide bonds such as dithiothreitol or 2-mercaptoethanol are also stressors.

The unfolded protein response is a generalized homeostatic adjustment, which is almost certainly transiently activated continually under normal physiological conditions. It is increasingly recognized as an important contributor to a wide range of conditions. Diseases associated with misfolded proteins are sometimes called conformational diseases. These include, particularly, neurodegenerative diseases such as Alzheimer’s, Parkinson’s, and prion diseases, which are associated with indicators of ER stress in the affected neural tissues. But the role of ER stress in the pathogenesis of such and other diseases is not clear because the problem of protein misfolding is not confined to the ER; cellular stress affects proteins in other organelles and compartments as well.

Other diseases involving ER stress include many, if not all, forms of ischemia-reperfusion injury, diabetes, obesity, inflammatory and autoimmune diseases, and liver disease. In cancer the ER is important for facilitating malignant cell survival under hypoxia. The unfolded protein response is stimulated, and PERK and XBP-1 are up-regulated, but the stress response is subverted to enable avoidance of apoptosis and tumor survival.

From the overview above, it is to be expected that ER stress responses will be identified in many forms of disease. In this issue of the *AJP*, Yung and colleagues demonstrate that protein synthesis inhibition and ER stress play key roles in the pathophysiology of intrauterine growth restriction. One should also consider the authors’ earlier study examining ER stress in the choriocarcinoma cell line JEG-3, which could be induced by deprivation of oxygen and glucose or by tunicamycin.

The authors singled out the survival kinase AKT (also known as protein kinase B) for detailed study. It is activated as a result of PI3-kinase activity in the cell membrane, where it is phosphorylated by phosphoinositide-dependent kinase 1 (PDK1). The gene family inhibits...
apoptosis, promotes cellular survival pathways, induces protein synthesis pathways, and is therefore a key signaling protein in the cellular pathways that lead to general tissue growth.\textsuperscript{15} In the JEG-3 cells subjected to ER stress, AKT protein was reduced, not because of lower transcription but because of reduced translation—the hallmark of ER stress. In the current study, these findings have now been extended by similar studies of placentas from pregnancies with unexplained fetal growth restriction (intrauterine growth restriction, IUGR), either in isolation or associated with preeclampsia.

Preeclampsia is an enigmatic problem of pregnancy that is easier to recognize than understand.\textsuperscript{16} It is defined in terms of its maternal syndrome (transient hypertension and proteinuria). However, the fact that it is attributable to the placenta has been long known. It is often associated with a fetal syndrome of IUGR, commoner in early onset preeclampsia than in that presenting at term. Uteroplacental arterial pathology underlies the problem. In the first half of pregnancy physiological dilatation of the terminal ends of the spiral arteries is impaired (poor placentation). This is linked to reduced invasion of the placental bed by extravillous cytotrophoblast. Lesions attributable to uteroplacental arterial insufficiency characteristicly include placent alinfarcts, more usually in early than late onset disease.\textsuperscript{17} But it has been argued that chronic placental hypoxia cannot explain all of the features of preeclampsia.\textsuperscript{18} Instead it is proposed that the critical pathology is hypoxia-reperfusion injury leading to acute oxidative stress. Indeed, there is evidence for intermittent blood flow in the intervillous space.\textsuperscript{18} In preeclampsia markers of placental oxidative stress are in general increased.

It is proposed that preeclampsia is a three stage disease: the primary pathology being an excessive or atypical maternal immune response to the conceptus shortly after implantation, leading to impaired placentation and oxidative stress in the placenta, and finally to the systemic maternal disease dysfunction.\textsuperscript{19} More is known about the role of placental hypoxia in the generation of the maternal syndrome of preeclampsia than of the mechanisms of fetal growth restriction. Hypoxia induces release of placental factors that, in the maternal circulation, dysregulate maternal endothelial function.\textsuperscript{20} The placental factors include increased anti-angiogenic proteins such as the soluble receptor of vascular-endothelial growth factor (sVEGFR-1) and endoglin A and reduced pro-angiogenic placental growth factor. The responses connect the hypoxic placenta with generalized maternal endothelial dysfunction in preeclampsia, a long recognized feature of the syndrome.\textsuperscript{21}

The phenotype of the preeclampsia placenta—its small size and the evidence for oxidative stress, for hypoxia-reperfusion and for increased apoptosis—\textsuperscript{22}—suggests that it should show ER stress. In the current study, Yung and colleagues\textsuperscript{1} show abnormalities primarily in the syncytiotrophoblast, where the ER is grossly dilated. This is both interesting and frustrating. Of all the cell types in the placenta, the syncytiotrophoblast is the most difficult to study in isolation. However, it can be argued that, because it forms the interface between mother and fetus, it is the most crucial to successful pregnancy. The dilated ER is most evident with severe stress in vitro gradations of stress induced by tunicamycin.\textsuperscript{1} The expected reconfiguration of protein synthesis and secretion (plasma cells and pancreatic \textit{beta} cells for example) are especially vulnerable to ER stress. Like wise, syncytiotrophoblast is a secretory tissue, perhaps explaining why it is more susceptible. Another question concerns its secretory functions. By what mechanisms and is associated with reduced cyclin D1, indicative of reduced cell proliferation.

As already mentioned, the authors previously found that one of the consequences of experimental ER stress is reduced availability of AKT, secondary to overall inhibition of protein synthesis. AKT takes the brakes off two important aspects of cell growth: mTOR (mammalian target of rapamycin), the master regulator of cell growth, which is otherwise restrained by tumor suppressor proteins hamartin (TSC1) and tuberin (TSC2), and glycogen synthase kinase 3 (GSK3). Each brake is released after AKT\textsuperscript{1}-induced phosphorylation, of tuberin and GSK3, respectively. GSK3 inhibits eIF2\textit{B} a heteropentamer and guanine nucleotide exchange factor that keeps eIF2 active. Removing these restraints promotes cell growth,\textsuperscript{23} and conversely, if AKT is less active the cell is directed toward inactivity. In the current study reduced AKT is also observed in IUGR placentas. This has the expected effects first on GSK3 signalling, which is reduced, and secondly on the activity of mTOR—two major pathways of cell growth. Downstream effectors of active mTOR are also diminished, except for the ribosomal protein S6-kinase1. Analogous findings are shown in Akt1 knockout mice, which are prone to IUGR.

In this study, they further develop the model of ER stress in choriocarcinoma JEG-3 cells to measure the consequences of \textit{in vitro} gradations of stress induced by tunicamycin.\textsuperscript{1} The expected reconfiguration of protein production is seen with increased levels of ER-resident chaperones GRP78 and GRP94 and of the apoptosis-inducing transcription factor CHOP. XBP-1 mRNA is spliced by the activated ER stress receptor and endonuclease IRE1, generating the activated transcription factor, which promotes production of UPR genes including GRP78 and GRP94. With greater ER stress, more JEG-3 cells die. Interestingly, activation of CHOP was only observed in the IUGR plus preeclampsia placentas, suggesting the ER stress is greater than in those from IUGR alone.

Which placental cells are the targets for ER stress? Yung and colleagues\textsuperscript{1} show abnormalities primarily in the syncytiotrophoblast, where the ER is grossly dilated. This is both interesting and frustrating. Of all of the cell types in the placenta, the syncytiotrophoblast is the most difficult to study in isolation. However, it can be argued that, because it forms the interface between mother and fetus, it is the most crucial to successful pregnancy. The dilated ER is most evident with severe stress in \textit{in vitro} experiments and with preeclampsia combined in IUGR in \textit{ex vivo} samples. Enlargement of the ER is a regulated response to ER stress, which is activated by XBP1.\textsuperscript{24}

Many questions are raised by this fascinating work. Whereas its focus is on the trophoblast, most of the placenta comprises other cell types such as stromal and endothelial cells, which apparently do not show the same degree of ER stress. Cells that specialize in protein synthesis and secretion (plasma cells and pancreatic \textit{beta} cells for example) are especially vulnerable to ER stress. Likewise, syncytiotrophoblast is a secretory tissue, perhaps explaining why it is more susceptible. Another question concerns its secretory functions. By what mechanisms
can the secretion of proteins such as leptin, sVEGFR-1, or corticotrophin-releasing hormone be enhanced in the preeclampsia trophoblast when it is subjected to protein synthesis inhibition secondary to ER stress? Or again, how are placental transport functions affected? Not all preeclampsia is associated with IUGR or placental pathology, so what is the status of placental ER in these presentations?

Much is now known of the tightly regulated internal organization of the cell and the inner workings of its organelles, such as the ER. Application of this knowledge to the trophoblast is only just beginning, with exciting prospects of future insights into human reproductive pathology.

References