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Survival by self-destruction: A role for autophagy in the placenta?☆

I. Bildirici [a,*], M.S. Longtine [b], B. Chen [b], D.M. Nelson [b]

[a] Department of Obstetrics and Gynecology, Acibadem University School of Medicine, Istanbul, Turkey
[b] Department of Obstetrics and Gynecology, Washington University School of Medicine, St Louis, MO 63110, USA

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ABSTRACT

Autophagy is a burgeoning area of research from yeast to humans. Although previously described as a death pathway, autophagy is now considered an important survival phenomenon in response to environmental stressors to which most organs are exposed.

Despite an ever expanding literature in non-placental cells, studies of autophagy in the placenta are lagging. We review the regulation of autophagy, summarize available placental studies of autophagy, and highlight potential areas for future research. We believe that such studies will yield novel insights into how placentas protect the survival of the species by "self-eating".

1. Introduction

Autophagy is Latin for "self eating", a term that now describes a vital biological phenomenon. Autophagy is a highly regulated process by which damaged proteins, injured organelles, and invading organisms are contained within double membrane-bound vesicles called autophagosomes that fuse with lysosomes, which leads to degradation of cargo in the resulting autolysosome to recycle biomolecules. A cassette of autophagy (Atg) genes were initially described in the yeast, *Saccharomyces cerevisiae*, and to date, 35 such genes are identified, with many conserved in species from slime mold to humans [1–6]. Apoptosis, autophagy, and necrosis were originally designated as cell death types I, II, and III, respectively [7]. However, this nomenclature is no longer pertinent, as autophagy is recognized to promote cell survival and rarely, does this cause cell death [8]. Moreover, autophagy declines in animal cells during aging *in vivo* and *in vitro*, linking the process to longevity [9,10]. Indeed, caloric restriction is both an inducer of autophagy and the only intervention known to promote longevity [11]. We below review the stressors for the induction, and the mechanisms for execution, of autophagy in mammalian cells. We next describe what little is known about autophagy in the placenta, we lastly highlight key areas for research as we opine that such research will shed light on the placental dysfunction associated with pregnancy maladies.

2. Induction and execution of autophagy

There are three types of autophagy. Microautophagy involves engulfment of cytoplasmic components by invaginations or protrusions of the lysosomal membrane [12]. In chaperone-mediated autophagy (CMA), a KFERQ-like pentapeptide in the target proteins is bound by a cytosolic chaperone, which results in translocation of the substrate to the lysosome for degradation [13]. Macroautophagy, designated hereafter as autophagy, is the focus of this review and involves delivery of cytosolic contents to lysosomes by autophagosomes. Table 1 summarizes mediators of the autophagy pathway.

Autophagy induction and cargo recognition leads to formation of the phagophore, a C-shaped double membrane organelle that sequesters cytoplasm or a specific organelle (Fig. 1). Closure of the phagophore around the cargo results in a double-membrane organelle, the autophagosome. The autophagosome then fuses with lysosomes via the help of the cytoskeleton. Fusion of the outer membrane of the autophagosome with the lysosomal membrane results in the entry of the inner membrane of the autophagosome into the membrane of the lysosome. Lysosomal enzymes degrade the inner membrane of the autophagosome and the cargo therein contained, releasing free fatty acids, monosaccharides, and amino acids into the cytoplasm for re-use.

Induction of autophagy requires an Unc-51-like kinase (ULK) complex that contains two related kinases, ULK1 and ULK2,

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* Corresponding author. Department of Obstetrics and Gynecology, Acibadem University School of Medicine, Gulsuyu Mah. Fevzi Cakmak Cad. Divan Sok. No:1, Maltepe, Istanbul, Turkey. Tel.: +90 2123044042; fax: +90 2122854584.
E-mail address: bildirici@superonline.com (I. Bildirici).

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Table 1
Mediators of autophagy.

Mediator	Alternate names	Function	References
Akt	Protein kinase B	Key downstream effector of the PI3K signaling pathway; regulates cell survival and proliferation. Akt inhibition leads to activation of autophagy. PI3K-Akt-mTOR pathway suppresses autophagy.	[101,102]
AMBRA 1	Activating molecule in Beclin 1 regulated autophagy	Regulates autophagy and development of the nervous system. AMBRA 1 binds to Beclin 1 and favors Beclin 1/VPS34 interaction.	[103–105]
AMPK	AMP-activated protein kinase	Senses reduced cellular energy level and regulates many aspects of cell function. AMPK inhibits mTOR-dependent signaling. It is required for autophagy in both yeast and mammalian cells. Activated AMPK triggers destruction of defective mitochondria (mitophagy), biogenesis of new mitochondria, and restrains cell growth and proliferation.	[106–108]
Bcl-2	B-Cell lymphoma 2 family of regulator proteins encoded by the-Bcl-2 gene	Dual regulator of apoptosis and autophagy; binds and sequesters Beclin 1. Dissociation of Beclin 1 from Bcl-2 is required for autophagy induction.	[69,109–111]
Beclin 1	Mammalian ortholog of the yeast Atg6 gene	Encodes a Bcl-2 interacting coiled-coil protein with autophagy and tumor suppressor function. Regulates kinase activity of VPS34.	[68,112–114]
BNIP3	Bcl-2/adenovirus E1b 19-kDa interacting protein 3	Competitively binds to Bcl-2 and releases Beclin 1, a known inducer of autophagy; specific activator of mitochondrial autophagy; inhibits mTOR activity and induces autophagy; crucial for hypoxia-induced autophagy	[54,115–119]
DAPK	Death-associated protein kinase	Phosphorylates Beclin 1 causing dissociation from Bcl-2 and Bcl-X_L which in turn induces autophagy; phosphorylates TSC2 leading to TSC1-TSC2 dissociation to stimulate mTOR in response to growth factor activation; plays a role in membrane blebbing.	[72,73,120]
DRAM	Damage-regulated autophagy modulator	This lysosomal protein is critical for p53 induction of autophagy and cell death, has a tumor-suppressive function.	[84,85,121,122]
ERK	Extracellular signal-regulated kinase	Depending on the cell type and stimulus, ERK activity will mediate different antiproliferative events, such as apoptosis, autophagy and senescence, *in vitro* and *in vivo*.	[123–127]
FOXO3	Forkhead Box O3	FoxO transcription factors are critical mediators of oxidative stress resistance in multiple cell types. FoxO transcription factors promote autophagy in skeletal muscle and have additional roles in regulation of cell size, proliferation, and metabolism. Decreased IGF-1-PI3K-Akt signaling activates autophagy not only through mTOR, but also by a transcription-dependent mechanism involving FoxO3.	[128–131]
Hif 1	Hypoxia inducible factor 1	Induces a cell survival response engaging autophagy mediated by BNIP3/BNIP3L	[50,132–134]
JNK	c-Jun N-terminal kinase	JNK activation modulates autophagy through two distinct mechanisms: 1. Promotes Bcl-2 phosphorylation resulting in the dissociation of the Beclin 1-Bcl-2 complex. 2. Upregulates DRAM, a p53 target gene.	[74,75,114,121,135]
LC3	Microtubule-associated protein Light Chain 3; Mammalian homolog of Atg8	Cytosolic form of LC3 (LC3-I) is conjugated to phosphatidylethanolamine to form LC3-II, which is recruited to autophagosomal membranes.	[136–139]
MCL-1	Myeloid leukemia cell differentiation protein	Has a key role in controlling both autophagy and apoptosis processes in a developmentally regulated manner.	[140,141]
mTOR	Mammalian target of rapamycin	Key negative regulator of autophagy; stress conditions and starvation inactivate mTOR.	[142–146]
PARP1	Poly (ADP-ribose) polymerase-1	Plays a dual role in the modulation of autophagy, apoptosis, and necrosis under oxidative stress	[61,147,148]
PI3K	Phosphatidylinositol 3-kinase	Class III PI3K regulates autophagy as well as protein synthesis through the mTOR pathway	[149–151]
p38	p38 mitogen-activated protein kinase	Plays a role in the control of autophagy at the sequestration and maturation step; regulates amino acid signaling, along with ERK 1/2 kinase	[127,152,153]
p53	Protein 53	Modulates autophagy in a dual fashion, depending on the sub-cellular localization in the nucleus versus the cytoplasm. Activation of p53 in the nucleus inhibits mTOR activity. The mechanisms by which p53 regulates mTOR activity in the cytoplasm involves AMP kinase activation and requires the tuberous sclerosis (TSC)1/TSC2 complex, both of which respond to energy deprivation in cells.	[84,146,154–158]
p62	Sequestosome	Links polyubiquitinated protein aggregates to the autophagy machinery; required for formation and activation of the caspase-8 complex	[82,83,159]
PTEN	Phosphatase and tensin homolog	Positively regulates autophagy by inhibiting the phosphatidylinositol 3-kinase/ protein kinase B pathway; antagonizes insulin dependent cell signaling; acts as a tumor suppressor gene through the action of its phosphatase protein product	[43,160]
Rag1 and 2	Ras-related GTPase	Small GTPases that regulate mTORC1 activity in response to amino acids, likely independently of TSC1/2 and Rheb.	[100]
Raptor	Regulatory associated protein of mTOR	mTOR partner in mTORC1. Binds substrates, including S6K1 and 4EBP1	[26,27]
Ras	Rat sarcoma protein	Small GTPase that acts as a negative regulator for nutrient deprivation induced autophagy through the Class I PI3K signaling pathway; activation mutations lead to malignant cell transformation	[37,38,161]
Rheb	Ras homolog enriched in brain	Small GTPase that regulates mTOR activity; regulates aggresome formation by inhibiting dynein dependent transportation of misfolded proteins	[162]
SIRT1	Sirtuin-1; NAD-dependent deacetylase sirtuin	SIRT1 can form a molecular complex with several essential components of the autophagy machinery, including autophagy genes Atg5, Atg7, and Atg8. SIRT1 can directly deacetylate these components, in an NAD-dependent fashion. Regulates longevity via autophagy.	[46,163,164]
TSC1/2 complex	Tuberous sclerosis complex Hamartin (TSC1) Tuberin (TSC2)	TSC1/2-Rheb-mTOR pathways play a central role in modulating cellular protein synthesis and autophagy.	[146,157]
ULK1 & 2	Unc-51-Like Kinase 1 &2 Mammalian Atg1 homologs	Formation of ULK complex (ULKs-Atg13-FIP200) plays an essential role in the initiation of autophagy	[14,165,166]

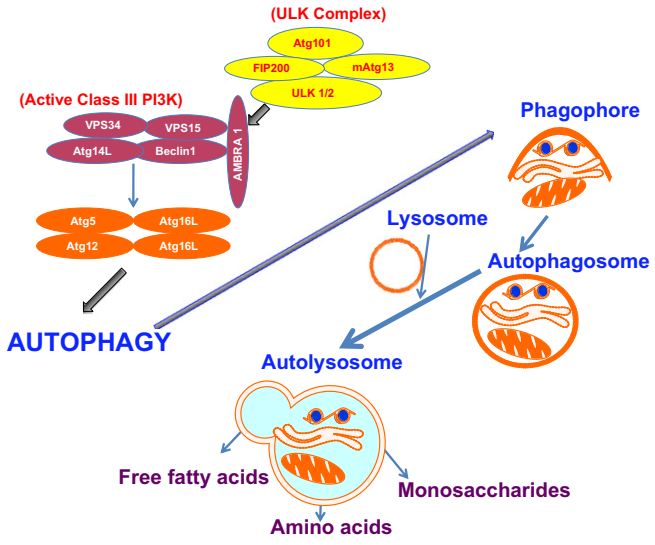

Fig. 1. Features of autophagy execution.

a scaffold protein, FIP200 (the focal adhesion kinase family interacting protein of 200 kDa), and mAtg13 [14–16]. Inactivation of mTOR yields dephosphorylation and activation of ULK1 and ULK2 and allows phosphorylation of FIP200 and mAtg13. The recently identified Atg101 binds and stabilizes Atg13 in the ULK-Atg13-FIP200 complex [17]. The nucleation and assembly of the initial phagophore membrane involves a class III phosphatidylinositol 3-complex (PI3K) consisting of the lipid kinase, vacuolar protein sorting 34 (VPS34), VPS15, Beclin 1, Atg14L and the activating molecule in Beclin 1-regulated autophagy (AMBRA 1) [18]. Phosphorylation of AMBRA 1 by ULK and association of Beclin 1 increase the PI3 kinase activity of VPS34. The activated PI3K complex results in the generation of phosphatidylinositol triphosphate (PI3P), which is essential for phagophore elongation and for the recruitment of heterodimers of Atg5 and Atg12, in combination with a homodimer of Atg16, to phagophore assembly sites. Membranes for the phagophore are initially derived from the ER, trans-Golgi network and late endosomes [19], but *de novo* synthesis of membranes is likely involved in the expansion of the phagophore. An ubiquitin-like system modulates the evolution of the autophagosomal membranes and yields the processing of microtubule-associated protein light chain 3-I (LC3-I) into the LC3-II isoform, which covalently attaches phosphatidylethanolamine. LC3 II is incorporated into phosphatidylethanolamine-rich lipid membranes during autophagosome genesis and thus, is commonly used as a specific marker for autophagosome formation [20]. Atg9 is an integral membrane protein, which can form multimers and which cycles between the autophagosomal membrane and other membranes. This may enhance phagophore expansion and phagophore tethering to the lysosome [21]. After phagophore expansion, closure and capture of cargo occurs, forming a mature autophagosome. Autophagosomes are trafficked to lysosomes upon microtubules by dynein motors, and en route, these may fuse with endosomes to form amphisomes. Fusion with lysosomes occurs, and the degradation of the lysosomal contents by hydrolases releases monosaccharides, amino acids, and free fatty acids into the cytoplasm for recycling.

3. Regulation of autophagy

Nutrient deprivation is a potent inducer of autophagy, and mTOR is at the center of nutrient sensing in cells. MTOR provides the catalytic subunit for two distinct multiprotein complexes, mTORC1 and mTORC2 (Fig. 2.), which share some proteins, e.g., GβL and Deptor, but have others, such as raptor in mTORC1 and rictor in mTORC2, that reflect the unique functions of each complex [22,23]. For example, raptor binds to mTOR signaling motifs on downstream targets, notably on ribosomal S6 protein kinase 1 (S6K1) and eukaryotic initiation factor 4-binding protein 1 (4EBP1). Proline-rich Akt substrate 40 kDa (PRAS40) associates with raptor to mediate the

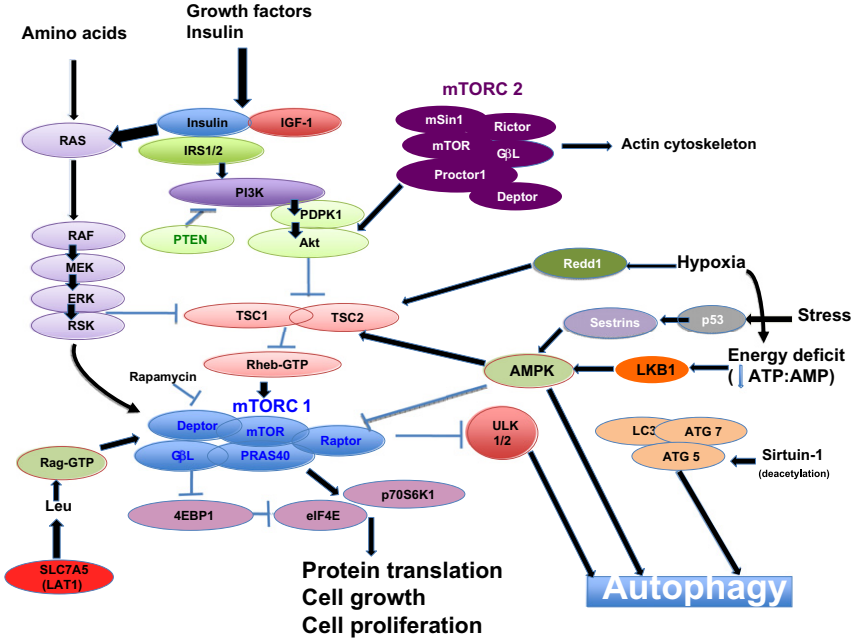

Fig. 2. Signaling pathways that regulate autophagy. Abbreviations not listed here are included in the text. Leu: Leucine mSin1: mammalian stress-activated protein kinase [SAPK]-interacting protein RAF, MEK and ERK (mitogen-activated protein kinase): serine/threonine selective protein kinases in the MAPK/ERK pathway. RSK: Ribosomal S6 kinase (activated by MAPK/ERK pathway) SLC7A5: Large neutral amino acids transporter small subunit 1; L-type amino acid transporter 1 (LAT1).

TOR signaling motif and this functions as a bridge downstream of mTORC1 and upstream of the effectors S6K1 and 4EBP1 [24–30]. Importantly, mTORC1 coordinates lipid and protein synthesis to enhance cell growth. Conversely, mTORC2 is implicated in the control of the actin cytoskeleton [31]. Inhibition of mTORC1 by rapamycin, for example, inhibits translation and activates ULK1/2 complex, which yields autophagy. An important regulator of mTOR is a small GTPase module consisting of Rheb, a small G-protein that activates mTORC1, and of the TSC1 (Hamartin)/TSC2 (Tuberin), which can stimulate Rheb GTPase activity [32].

Sufficiency of amino acids, energy, and growth factors promote mTORC1, whereas regulation of mTORC2 remains mostly unknown [33]. In HeLa cells, bidirectional transport of glutamine out of the cell and leucine into the cell by large neutral amino acids transporter small subunit 1 (SLC7A5 or LAT-1) induce phosphorylation of mTORC1 and inhibit autophagy. In skeletal muscle, increased availability of essential amino acids is shown to both stimulate mTORC1 signaling and increase amino acid transporter expression [34,35]. In the presence of amino acids, Rag proteins, the Ras-related small GTPases, also activate mTOR and inhibit autophagy [36]. Conversely, limited availability of these amino acids inhibits mTORC1 and induces autophagy.

In yeast, Ras/cAMP-dependent protein kinase A (PKA) signaling pathway plays an important role in glucose sensing and negatively regulates autophagy in parallel with the mTOR pathway [37,38]. In nutrient-rich conditions, the small GTPases, Ras 1 and Ras 2 are active and enhance cAMP generation, resulting in activation of protein kinase A (PKA), with downstream suppression of TSC and activation of mTORC1.

Growth factors suppress TSC function by both PI3K-dependent and independent pathways. The latter occurs via Ras and MEK downstream pathways with activation of the mTORC1 signaling [32]. Insulin and insulin-like growth factors (IGF) regulate mTOR through the Class I PI3K. Insulin binding to the insulin receptor induces autophosphorylation, and recruitment of insulin receptor substrates (IRS) 1 and 2, where PI3K binds to yield phosphatidylinositol (3,4,5)-triphosphate (PIP_3), increases membrane recruitment of protein kinase B (Akt) and phosphorylation of phosphoinositide-dependent protein kinase 1 (PDPK1). PDPK1 phosphorylates Akt which in turn phosphorylates TSC2, blocking its interaction with TSC1 [39] and thereby allowing GTP-Rheb to bind and maintain active mTORC1, inhibiting autophagy [40–42]. Conversely, the 3′-phosphoinositide phosphatase PTEN reverses PIP_3 production, decreasing Akt signaling and activating autophagy [43].

Phosphorylation and deacetylation are among important post-translational modifications for the regulation of autophagy. Recently, specific mTOR phosphorylation sites such as Ser^{1261} Ser^{2159} and Thr^{2164} that regulate mTORC1 function were identified [44,45]. The NAD-dependent deacetylase sirtuin-1 forms a complex with several proteins of the autophagy machinery and deacetylates them to allow autophagy to proceed [46].

Energy levels are detected through the 5′-AMP-activated protein kinase (AMPK), which senses reduced cellular ATP levels. A decreased ATP/AMP ratio activates AMPK through the upstream LKB1 kinase. AMPK then phosphorylates the TSC1/2 complex, which promotes GTP-Rheb activity, inhibiting mTOR activity [47]. AMPK also inhibits mTOR by directly phosphorylating raptor [48]. Unlike necrosis or apoptosis, cellular ATP production increases throughout the autophagic process that recycles nutrients. Moreover, the LKB1-AMPK pathway activates the cyclin-dependent kinase inhibitor, $P27^{kip1}$, leading to cell-cycle arrest [49]. The end result of the induced autophagy is survival through partial self-destruction, cell cycle arrest, and avoidance of apoptotic cell death, despite the presence of nutrient deprivation.

Hypoxia induces autophagy by at least two mechanisms. First, prolonged hypoxia reduces ATP levels and thus activates AMPK. Secondly, hypoxia stabilizes hypoxia-inducible factor 1 (Hif 1), a transcription factor that is a major regulator of the cellular response to hypoxia [50]. Increased expression of Redd1 in hypoxia which is in part due to transcription activity by Hif 1, activates TSC1/2, suppresses mTORC1 activity and results in autophagy [51]. Hif 1 also induces transcription of Bcl-2/adenovirus E1a 19-kDa interacting protein 3 (BNIP3), and the related BNIP3L [52–54]. Bnip3/Bnip3L binds Bcl-2 and Bcl-XL, disrupting the interaction with Beclin, allowing Beclin to associate with the class I PI3K complex and activate autophagy. Notably, low oxygen levels play a vital role in early placental development, as invasive trophoblast normally differentiate in a $pO_2 < 20$ mm Hg [55]. However, by 10–12 weeks of gestation, oxygen delivery to the intervillous space by maternal spiral arterioles raises the pO_2 to about 60 mm Hg, and the developing fetus is dependent upon adequate placental oxygen delivery for the remainder of pregnancy.

Oxidative stress plays a major role in the induction of autophagy, especially by generation of superoxide anions [56,57]. The electron transport system that provides energy from mitochondria generates multiple reactive oxygen species (ROS) and hydrogen peroxide, all of which must be eliminated by cell anti-oxidant defenses to avoid cellular damage. When cell oxidant levels exceed anti-oxidant defenses, lipids, proteins, DNA, mitochondria, and other organelles are damaged. Induction of autophagy can eliminate these damaged components. Induced mitophagy eliminates damaged mitochondria, reducing ROS generation and contributing to cell survival [58,59]. The cysteine protease Atg4 cleaves Atg8/LC3 from the autophagosome outer membrane. ROS inhibit Atg4 protease activity and enhance lipidation of Atg8/LC3, which is essential for autophagy [60]. Atg4 and ROS through the activity of poly (ADP-ribose) polymerase-1 (PARP-1) stimulates the LKB1-AMPK pathway to induce autophagy [61].

4. Crosstalk between apoptosis and autophagy

Autophagy and apoptosis are both important responses to cellular stress [62–64]. Apoptosis and autophagy can act cooperatively to yield cell death, but recent data show that autophagy often promotes cell survival by antagonizing apoptosis. Not surprisingly, cross talk between the two phenomena is extensive, as recently reviewed [65–67]. There has been significant progress in the identification of mediators that participate in both autophagy and apoptosis.

Notably, Beclin 1, identified as Atg6, was found to be a binding partner for Bcl-2, the first identified member of a family of proteins that controls apoptosis [68]. Bcl-2 family members play opposing roles in the regulation of apoptosis and autophagy [69], while Beclin 1 is a key regulator of autophagy. The anti-apoptotic proteins Bcl-2 and Bcl-XL can each bind to a BH3 domain in Beclin 1 to inhibit autophagy [70], especially when nutrients are plentiful [71]. When nutrients are depleted, JNK1 phosphorylates Bcl-2, Death Associated Protein Kinase (DAPK) phosphorylates Beclin 1 and both phosphorylations facilitate Bcl-2 separation from Beclin 1, the latter activating autophagy through interaction with AMBRA 1, VPS15 and 34 [72,73]. During prolonged starvation, the phosphorylated Bcl-2 binds to the proapoptotic protein Bax to inhibit apoptosis, but when survival is no longer possible, JNK1 mediates hyperphosphorylation of Bcl-2, Bax detaches, and apoptosis ensues [74,75]. Atg5 is cleaved by calpains during apoptosis and cleaved Atg5 interacts with Bcl-XL in mitochondria to control apoptosis. The absence of mitochondrial Atg5 yields autophagy [76].

Flice-inhibitory protein (FLIP) plays a double role, as a negative regulator of the extrinsic pathway to apoptosis and as an inhibitor

of autophagy by blocking Atg3 conjugation to LC3 [77]. If caspases are activated, they not only degrade apoptotic substrates but also degrade autophagic proteins such as Beclin 1 to inhibit autophagy and potentiate apoptosis [78–80]. Conversely, cell survival is promoted through autophagy when Beclin 1 physically interacts with survivin to prolong the function of this inhibitor of apoptosis [81].

Sequestosome (p62) is a multifunctional protein that binds to Atg8/LC3 yielding proteasome activity and autophagy [82]. Importantly, p62 is also required for formation and full activation of the caspase-8 complex, important as an initiator caspase in the extrinsic pathway [83]. Moreover, LC3-II binds p62 to deliver misfolded proteins and injured organelles to the autophagosome for degradation.

P53 is a pivotal protein in effecting apoptosis and this protein regulates autophagy in a bidirectional manner, depending upon the sub-cellular location of p53 action. Cytoplasmic p53 blocks autophagy while nuclear p53 functions in transcriptional regulation of sestrins (SES) 1 and 2 and provides these proteins to phosphorylate AMP-responsive protein kinase (AMPK), which in turn phosphorylates TSC2 and activates autophagy. Other nuclear effects of p53 include increased IGF-BP3, PTEN, and ARF. Interestingly, damage-regulated autophagy modulator (DRAM) is also essential in p53-mediated apoptosis further illustrating the intertwining of autophagy and apoptosis [84,85]. P53 promotes phosphorylation of Bcl-2, which dissociates the latter protein from Beclin 1 to enhance autophagy. P53 regulates DRAM expression and this lysosomal protein also enhances autophagy. Conversely, the p53-induced glycolysis and apoptosis regulator (TIGAR) inhibits glycolysis and decreases cellular ROS levels to inhibit autophagy [86]. The complexity of p53 regulation of autophagy is intimately related to the activity of p53 in apoptosis.

5. Autophagy in placenta

A role for autophagy during the trophoblast interactions with the endometrium in early implantation was implicated more than 35 years ago [87]. However, only a few studies to date focus on autophagy in placental function. Approximately 15 years ago, APG9L2, a mammalian homolog of the first identified autophagy protein (Atg9p) in yeast, was shown to be differentially expressed in the placenta and pituitary gland but not other tissues, while another homolog APG9L1 was ubiquitously expressed in adult human tissues [88]. Notably, APG9L2 showed a four-fold decrease in expression in syncytiotrophoblast compared to cytotrophoblast, suggesting a developmental regulation during trophoblastic differentiation.

Autophagy occurs in trophoblast from term placental villi, with a higher level of autophagy in villi from cesarean section placentas reflected by accumulation of more LC3-II, compared to placentas from spontaneous vaginal delivery [89]. The lower cord blood glucose levels of infants delivered by cesarean section, from maternal fasting prior to the operation, is suggested to be the etiology of this counterintuitive proposition. The result may reflect the fact that autophagy is activated as a survival mechanism during nutrient deprivation [89]. Roh and colleagues [90] showed increased expression of LC3-II, but not Beclin 1, in placentas of women with severe preeclampsia, compared to controls. In the JEG-3 choriocarcinoma cell line, hypoxia resulted in increased LC3-II and diminished Beclin 1. Interestingly, TNF-α is also a known inducer of apoptosis in primary human trophoblasts [91] and this cytokine increases LC3-II expression in the JEG cells [90].

The mTOR system is a pivotal regulator of autophagy, as described above, and this nutrient sensing system has recently been studied in primary human trophoblasts [92]. Glucose deprivation down-regulates system L activity in an mTOR dependent manner in cultures of trophoblast [92]. Conversely, IGF-1 increases system A and insulin increases system L amino acid transport activity, both in an mTOR dependent manner [92]. MTOR signaling regulates the activity of key amino acid transporters [93] and both mTOR signaling and placental amino acid transporters are down regulated by maternal protein deprivation [94]. Interestingly, placentas from pregnancies complicated by IUGR have reduced mTOR activity [95,96].

A recent study found that, in addition to mTOR, human placental villous trophoblasts express Deptor, Raptor, and Rictor, other components of mTORC1. Interestingly, Deptor expression is reduced in placental villi exposed to maternal stress *in vivo* and in trophoblast cell lines exposed to the stress hormone cortisol. Thus, maternal stress may influence autophagy in placenta [97].

An interesting study by Hung and colleagues [98] noted concomitant exposure to vitamins C and E differentially affected trophoblast apoptosis and autophagy in villous explants under conditions simulating normoxia and hypoxia with re-oxygenation *in vivo*. Specifically, vitamins C and E reduced the levels of apoptotic markers under normoxic conditions but increased apoptosis when hypoxia re-oxygenation occurred. Moreover, vitamin treatment led to reduced LC3 immunostaining and reduced LC3B expression on immunoblot analysis, in explants exposed to 8% oxygen to simulate normoxic conditions. Conversely, there was increased expression of LC3B-II in both trophoblast and stromal cells in hypoxia re-oxygenation conditions when vitamin C and E were present, but not in the 8% oxygen paradigm. Notably, cultured cytotrophoblasts treated with vitamins C and E under hypoxia re-oxygenation conditions showed decreased expression of the anti-apoptotic proteins, Bcl-2 and Bcl-XL, increased expression of proapoptotic Bak, enhanced cytosolic LC3-II and decreased interaction of Beclin 1 with Bcl-2 and the Bcl-XL/Beclin 1 complex. These results are consistent with enhanced autophagy under conditions of re-oxygenation [98]. These studies suggest that insults to placental villi and trophoblast cells yield condition-specific responses in the balance between trophoblast autophagy and apoptosis.

The importance of placental autophagy in fetal development and survival is underscored by effects of maternal starvation in a mouse model of the imprinted gene PEG 3 (paternally expressed gene 3) [99]. Undernutrition in mid-gestation desynchronizes the expression of PEG 3, increasing PEG 3 expression in fetal hypothalamus and decreasing PEG 3 expression in placenta. This desynchronization results in enhanced placental autophagy, specifically ribophagy, which produces nutrients to sustain energy demands of the hypothalamus. These data suggest that the fetus in general and the fetal brain in particular is spared during short-term acute maternal starvation, at the expense of the placenta.

6. Future research on autophagy and the placenta

The regulation of autophagy and the mechanisms involved with induction in non-placental tissues have made substantial strides in the last decade. The role mTOR in nutrient sensing of the placenta is established, but what role this protein plays in the balance of trophoblast, and thus fetal survival and demise is unknown. Moreover, autophagy has only recently been identified in human placentas. Several key questions must be addressed. First, what role does autophagy play in trophoblast homeostasis? Does autophagy contribute to placental dysfunction in the maladies of pregnancy? Alternatively, does autophagy provide protection for placentas exposed to exogenous stressors during pregnancy, perhaps by inhibiting apoptosis and aiding in nutrient recycling? What is the interplay of autophagy and apoptosis in the trophoblast layer,

particularly in complicated pregnancies? We speculate that future research will identify key roles for autophagy in placental function of women with preeclampsia, IUGR, or both. We also predict that autophagy will be pivotal in the response of trophoblasts to stressors present in normal pregnancies, allowing the trophoblast, in particular, and placental villi in general, to adapt to the variety of insults normally encountered in pregnant women. We believe that self-destruction by autophagy has a unique mission to protect placental function during stress and thereby, to optimize fetal development. We propose that studies evaluating the regulation of autophagy in the placenta will offer new insights into placental function under homeostatic conditions and the placental dysfunction that accompanies exposure to exogenous insults.

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