Chapter 16

The Role of Autophagy in Maintaining Pregnancy

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Abstract

Autophagy is an evolutionarily conserved process in eukaryotes by which cytoplasmic cargo sequestered inside double-membrane vesicles is delivered to the lysosome for degradation. In early pregnancy, trophoblasts and the fetus experience hypoxic and low-nutrient conditions; nevertheless, extravillous trophoblasts (EVTs) invade the uterine myometrium up to one-third of its depth and migrate along the lumina of spiral arterioles, replacing the maternal endothelial lining. An enhancement of autophagy induced by physiological hypoxia takes part in the invasion and vascular remodeling in EVTs. On the other hand, soluble endoglin, which increased in sera in preeclamptic cases, suppresses EVT-invasion or -vascular remodeling by inhibiting autophagy *In vitro*. In addition, a substance selectively degraded by autophagy, p62/SQSTM1, accumulates in EVT cells in preeclamptic placental biopsy samples showing impaired autophagy *in vivo*. Thus, alternation of autophagy could affect fates of mothers and babies. Recently increasing evidence of modulating autophagy has accumulated during pregnancy. In this chapter, we introduce the role of autophagy in embryogenesis, implantation, and maintaining pregnancy.

Keywords: autophagy, extravillous trophoblast, hypoxia, invasion, preeclampsia, p62/SQSTM1, soluble endoglin

1. Introduction

The placenta acts to mediate the exchange of materials between mother and fetus under hypoxic and low-nutrient conditions during the early gestation period [1, 2]. It has been reported that hypoxia and low nutrient, which are generally harmful for cells, are preferable

for trophoblasts during the early gestation period [3–5], indicating that trophoblasts possess evolutionary mechanisms allowing them to adjust to stress. In other words, disruption of these adaptive mechanisms may contribute to placental dysfunction, which induces preeclampsia and fetal growth restriction (FGR). Preeclampsia is one of the important diseases for life threatening a baby as well as a mother. It also causes FGR. Ten million women develop preeclampsia each year, and 76,000 mothers among them die each year all over the world. A recent interest for autophagy researchers is how autophagy contributes to the human diseases. It is getting clarified the role of autophagy for pathophysiology of preeclampsia or pregnancy-related diseases. In this chapter, we focus on the role of autophagy as a cellular cytoprotective mechanism, especially for embryogenesis, implantation, and maintaining pregnancy.

2. Autophagy in embryogenesis and implantation

Macroautophagy (herein referred to as autophagy) has long been considered a nonselective process for bulk degradation of either long-lived proteins, or cytoplasmic components during nutrient deprivation. Autophagy works for not only cellular energy metabolism, but also quality control for cellular protein (by eliminating protein aggregates, damaged organelles, lipid droplets, and intracellular pathogens) [6]. Though lysosomal degradation is served as a final step of autophagic machinery, this machinery can be deployed in some cellular processes: phagocytosis, exocytosis, secretion, antigen presentation, and regulation of inflammatory signaling [7]. Consequently, the autophagy pathway is mediated with human diseases, such as protection against aging, suppression or development of cancers, infections, neurodegenerative disorders, metabolic diseases, inflammatory diseases, and muscle diseases [8–12].

Autophagy has a variety of functions during embryogenesis (**Figure 1**). Autophagy is highly induced in fertilized oocytes, but not unfertilized oocytes, within 4 hours after fertilization [13]. As oocytes lacking Atg5 are fertilized normally *in vivo*, autophagy is not important for folliculogenesis and oogenesis. Similarly, ovulation, fertilization, implantation, and ovary size are not affected by the ablation of Beclin1 (BECN1) in luteal cells of ovary [14]. During oocyte-to-embryo transition, many of maternal proteins stored in oocytes are provided to zygotes (fertilized embryos), the stock of these proteins is largely degraded, and newly synthesized proteins encoded by the zygotic genome are translated. In mice, it is known that zygotic transcripts are detected at the late one-cell stage, and most of the maternal RNAs are eliminated by the two-cell stage [15–17]. During this period, autophagy is transiently suppressed from the late one-cell to middle two-cell stages, and reactivated after the late two-cell stage, suggesting that autophagy is involved in the degradation of maternal proteins in oocytes. In addition, complete autophagy-deficient embryos, which are derived from oocytes lacking Atg5 fertilized with Atg5-null sperm, arrest at four- to eight-cell stages, but not embryos derived from oocytes with Atg5-plus sperm [13]. Protein synthesis rates are reduced in complete autophagy-deficient embryos, suggesting that the degradation of maternal factors by autophagy is essential for preimplantation embryo development in mammals.

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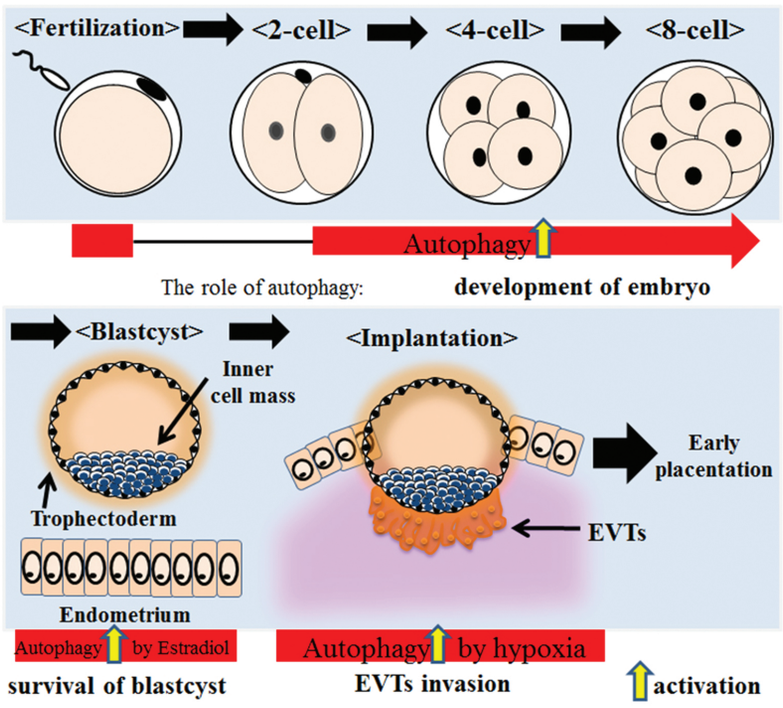

Figure 1. The role of autophagy during embryogenesis: Activation of autophagy occurs from the late two-cell stage to the eight-cell stage. Autophagy helps survival of blastocysts treated with estradiol (E2). Subsequently, EVTs' invasion, which is necessary for normal placentation, is supported by hypoxia-activated autophagy.

A mouse model of experimentally delayed implantation, established by ovariectomy before blastocyst implantation, showed that Atg7 and LC3 expression is upregulated in blastocysts made dormant by the elimination of 17β-estradiol (E2), in comparison with E2-activated blastocysts, suggesting that autophagy is sustained during the prolonged survival of dormant blastocysts [18]. Activation of autophagy is also observed in the inner cell mass, known as the embryoblast or pluriblast, which will evolutionally give rise to the definitive structures of the fetus. On the other hand, E2 or progesterone activates autophagy simultaneously with a decrease in activation/phosphorylation of mTOR (mammalian target of rapamycin) in bovine mammary epithelial cells [19].

Mice lacking BECN1, which is necessary for autophagy [20], died for early embryonic period (E7.5 or earlier) with defects in proamniotic canal closure [21]. Ablation of FIP200 (focal adhesion kinase family interacting protein of 200 kD) in mice also leads to embryonic lethality at mid/late gestation, which is caused by defective heart and liver development [22]. Most mouse embryos with functional deficiency of Ambra1 (activating molecule in beclin1-regulated autophagy) exhibit neural tube defects in midbrain/hindbrain exencephaly and/or spina bifida during E10–E14.5 [23]. Therefore, these types of autophagy deficiency are mainly involved in the impairment of embryonal development. However, it is unknown whether autophagy deficiency affects placental development or physiological status in dams.

Mice lacking Atg5 are born normally, but die within the first day after birth [24]. Atg7, which is essential for Atg5-Atg12 conjugation, knockout mice are also born at expected ratios, with

large healthy major organs. Similar to Atg5 knockout mice, Atg7 knockout mice also die earlier than wild-type controls under nonsuckling conditions after cesarean delivery, and have lower plasma amino acid levels [25]. Atg3, Atg9, and Atg16L1 conventional knockout mice demonstrate similar phenotypes [26–28]. The exact reason for neonatal death in those knockout mice is still controversial, and observation of a suckling defect may indicate a neuronal defect. However, the above studies demonstrate that amino acid supply through autophagy is important for mouse neonatal survival, preventing sudden starvation at birth. In addition, autophagy knockout mice that are born normally exhibit slightly inhibited fetal growth. It is still unknown whether inhibited growth is a result of defects within the placenta, or by mechanisms within the pups themselves. This question could be elucidated with the use of placenta-specific autophagy-deficient mouse. The BECN1-containing PI(3)K and ULK1-FIP200 complexes function early in the autophagy process at the autophagosome nucleation step [9, 29], while Atg3, Atg5, Atg7, and Atg16L1 function later at autophagosome elongation. As shown in **Table 1**, genetic knockout upstream factors may produce more severe phenotypes, or alternatively, as recently reported, downstream factors may be responsible for a particular type of macroautophagy [30]. In fact, LC3B knockout pups survive longer than their wild-type counterparts without a compensatory increase in LC3A, demonstrating opposite results to the other Atg-knockout mice [31]. Atg4C, a family of cysteine proteinases for processing and delipidation of Atg8, knockout mice also indicate that Atg4C is dispensable for embryonic and adult mouse development, as well as for normal growth and fertility [32].

Genes	Phenotypes
$Atg3^{-/-}$, $Atg5^{-/-}$, $Atg7^{-/-}$, $Atg9^{-/-}$, $Atg16L1^{-/-}$	Neonatal lethal with reduced amino acid levels, suckling defect (Atg5: the failure of the four- and eight-cell stages in embryogenesis)
Beclin $1^{-/-}$	Early embryonic lethal (E7.5 or earlier) with defects in proamniotic canal closure (heterozygous mice show increased susceptibility to spontaneous tumor)
$FIP200^{-/-}$	Embryonic lethal (E13.5–E16.5) dueto defective heart and liver development
$Ambral^{gt/gt}$	Embryonic lethal (-E14) with defects in neural tube development, and hyperproliferation of neural tissues
$ULK1^{-/-}$	Increased reticulocyte number with delayed mitochondrial clearance
$Atg4C^{-/-}$	Viable, fertile, increased susceptibility to carcinogen-induced fibrosarcoma
$LC3B^{-/-}$	Normal phenotype, production higher levels of IL-1β and IL-18 in response to LPS
$GABARAP^{-/-}$	Normal phenotype

Table 1. Phenotypes of systemic knockout mice of Atg-related genes.

3. The role of autophagy in trophoblasts for normal development of the placenta

In humans, trophoblast stem cells differentiate into two cell types: villous trophoblasts and extravillous trophoblasts (EVTs). Invading trophoblasts called interstitial EVTs migrate into

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the decidualized endometrium, and endovascular EVTs migrate along the lumina of spiral arterioles. The invasion of spiral arteries by EVTs starts early in pregnancy, and the endovascular trophoblastic cells aggregate in the lumen of the vessel forming the "trophoblastic plug" to allow the growth of the embryo and the placenta in a low-oxygen environment (**Figure 2**). As the EVTs migrate away from the villi and invade the maternal decidua, they progressively develop an invasive phenotype without proliferation [33], and stop at one-third of the depth of the myometrium in the uterus. EVTs invade the maternal decidua under harsh conditions, including low oxygen (2–5% O_2) and low-glucose concentrations (1 mM), until 11 weeks of gestation [33, 34]. During the invasion, the hypoxia inducible factor (HIF) system plays a critical role in their functions. After 12 weeks of gestation, endovascular EVTs invade the uterine spiral arteries, replace their endothelial cells, and participate in the degradation of tunica media smooth muscle cells under moderate hypoxia (approximately 8%). This remodeling of the spiral arteries is essential to allow a proper placental perfusion to sustain fetal growth (**Figure 2**). In other words, impairment of trophoblast invasion or remodeling may contribute to fetal loss during early pregnancy, or poor placentation during the middle of the pregnancy period.

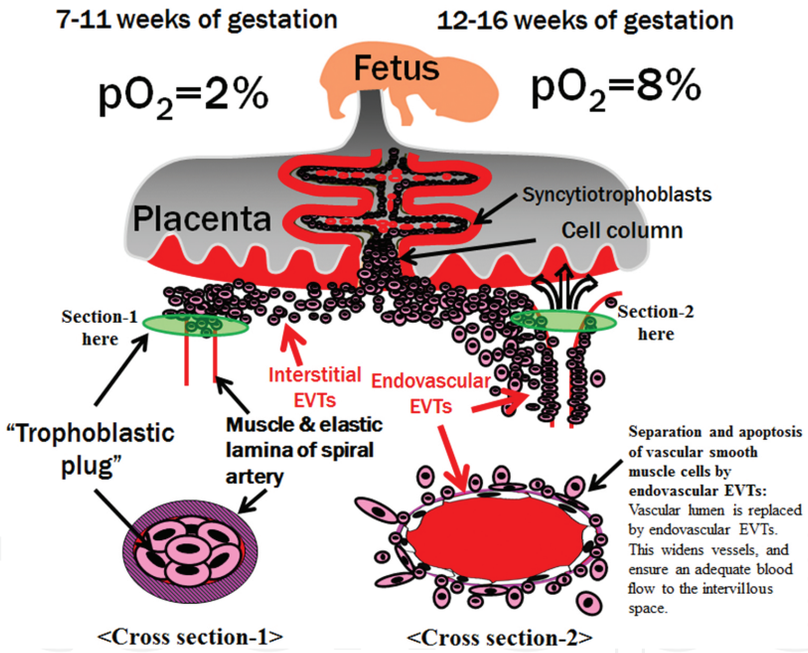

Figure 2. Autophagy supports EVT functions under physiological hypoxia: Interstitial EVT invades during 7–11 weeks of gestation, and vascular remodeling by EVTs occurs during 12–16 weeks of gestation. Cross section-1 indicates the "trophoplastic plug" to maintain hypoxia in the conceptus during 7–11 weeks of gestation. Cross section-2 indicates separation and apoptosis of vascular smooth muscle cells by endovascular EVTs.

Activation of autophagy is observed in EVTs invading into the maternal decidua at 7 weeks of gestation, under physiological hypoxic conditions [35]. Hypoxia induces autophagy in primary trophoblasts [35, 36], and inhibition of autophagy induced by silencing Atg7 in primary trophoblasts decreases apoptosis under 1% oxygen conditions in the presence of bafilomycin A1, an inhibitor of lysosome, indicating that autophagy mediates apoptosis in

trophoblasts under specific conditions. Not only hypoxia, but also starvation induces autophagy in trophoblasts or some choriocarcinoma cell lines, and LC3-II/actin levels, a marker of autophagic activation, vary depending on the cell lines (unpublished data). Though many independent studies have shown that hypoxia enhances invasion of EVTs, the role of autophagy in trophoblast functions is still unclear. To elucidate the specific role of autophagy in trophoblast functions, we constructed autophagy-suppressed cells by stably transfecting $ATG4B^{C74A}$, an inactive mutant of ATG4B, which inhibits autophagic degradation and lipidation of LC3B paralogs [37]. In these cell lines, the conversion of LC3-I to LC3-II was abolished by starvation, a commonly used autophagy stimulator. Hypoxia enhances the invasive capacity of EVT cell lines, such as HTR-8/SVneo, the most widely used EVT cell line, or HchEpC1b, immortalized by infection with retroviral expression vectors containing the type 16 human papillomaviruses E6 and E7 in combination with a human telomerase reverse transcriptase, with a normal chromosomal number and no tumorigenic activity [38]. The invasion was significantly reduced in autophagy-suppressed EVT cell lines, compared with autophagy-normal EVT cell lines, under 2% oxygen tension, which matches the placental condition until 11 weeks of gestation.

The other function of EVTs, vascular remodeling, is also necessary to precisely develop placentation. To clarify the role of autophagy in vascular remodeling, tube formation assays with EVT cells and human umbilical vascular endothelial cells (HUVECs), an in vitro model of vascular remodeling by EVT cells, were performed under 8% oxygen tension, simulating physiological levels of oxygen tension after 12 weeks of gestation [39]. Both the autophagy-normal EVT cells and the autophagy-suppressed cells formed a tube structure with HUVECs at 12 h, but did not form a tube structure in the absence of HUVECs. In the culture with the autophagy-normal EVT cells and HUVECs, the tubes were mostly occupied by the autophagy-normal EVT cells at 12 h or later; whereas the tubes were not occupied by EVT cells when the autophagy-suppressed cells were cocultured with HUVECs, suggesting that replacement of endothelial cells by EVT cells requires autophagy. Thus, autophagy plays an important role in the endovascular interaction between EVT and endothelial cells [35].

No difference in HIF1α expression was observed between autophagy-normal and autophagy-suppressed EVT cell lines [35], suggesting that HIF1α is not affected by autophagy status. A number of studies have investigated the role of the HIF1 pathway in EVT invasion. A decrease in HIF1α expression induced by siRNA markedly reduced the invasiveness of HTR8/SVneo cells under hypoxia and normoxia [40]. Hypoxia-induced autophagy is modulated by the inactivation of mTOR via AMPK (5′-AMP-activated protein kinase) [41]. Additionally, Atg5 knockout, but not wild type, mouse embryo fibroblasts (MEFs) showed no activation of autophagy under hypoxia [42]. Thus, HIF1α may activate EVT functions, at least partially, by manipulating autophagy status. Rapamycin or siRNA-mediated mTOR knockdown, an activator of autophagy, reduced the invasiveness of HTR8/SVneo cells under normoxia [43]. However, our study revealed no effect of rapamycin on invasion of HTR8/SVneo cells [44]. This result might be explained by a rapamycin-induced cell cycle arrest in the G1 phase [45].

Hypoxia activates macroautophagy via the HIF1 pathway, and HIF1α seems to be controlled by autophagy. Chaperone-mediated autophagy (CMA) has recently shown to degrade

HIF1α, which is mostly controlled by the oxygen-dependent proteasome, through LAMP2A, a lysosomal transporter protein. Interestingly, this new pathway for degradation of HIF1α does not depend on the presence of oxygen and is activated in response to nutrient deprivation in rat livers [46]. CMA-mediated excessive degradation of HIF1α compromises cells' ability to respond to and survive under hypoxia, suggesting that the impairment of this pathway might be of pathophysiological importance in conditions that combine hypoxia with starvation, including the early pregnancy period, during which EVTs invade.

Placenta expresses LC3B in both cytotrophoblasts and syncytiotrophoblasts. LC3-II/actin levels, which indicate activation of autophagy, are higher in placentas from cesarean sections than from vaginal deliveries [47], suggesting that uterine contraction might inhibit autophagy in placentas. In other words, autophagy might be activated in normal placenta before delivery. Autophagic cell death is also reported in amniotic epithelium following the rupture of membranes in term placentas [48]. Atg9L2 (Atg9b) is specifically expressed in placenta (trophoblast cells) and pituitary gland, while another homolog Atg9L1 (Atg9a) is ubiquitously expressed in adult human tissues [49]. Atg9L1 and Atg9L2 are involved in autophagosome formation [26]. Mouse Atg9L2 is found to be more widely expressed at embryonic stages than in adulthood. In humans, the expression of Atg9L2 is significantly higher than that of Atg9L1 in human primary cytotrophoblasts, suggesting that Atg9L2 contributes to tissue-specific and developmental activation of autophagy. Autophagy-deficient mice, similar to Atg7 or Atg5 knockout mice, are born at expected ratios, and fetal weight is only slightly lower than that of wild-type fetuses. Kojima et al. [50] recently reported the role of autophagy in preeclampsia in $Atg9a^{-/-}$ mice mated with heterozygous $p57^{Kip2}$ mice, which develop hypertension and proteinuria in dams. Fetal death was increased in $Atg9a^{-/-}$ and $Atg9a^{+/-}$ pups, compared with wild-type controls [51]. In addition, the body weight of fetuses in $Atg9a^{-/-}$ pups was significantly lower than those of $Atg9a^{+/-}$ or wild-type pups, suggesting that autophagy sustains fetal spare ability under stress. A future task is to clarify the role of Atg9b, which is highly expressed in the placenta.

4. The role of autophagy in the pathophysiology of preeclampsia

The current hypothesis regarding the etiology of preeclampsia is focused on shallow trophoblast invasion and poor placentation [52, 53] (**Figure 3**). We recently reported that autophagy was enhanced in EVTs in early gestation placental tissues, which are under physiological hypoxia [35]. As mentioned previously, the impairment of the invasion and vascular remodeling under hypoxia, which are thought to be a cause of preeclampsia, are observed in autophagy-deficient EVT cell lines. Furthermore, soluble endoglin (sENG), levels of which increase in sera before the onset of preeclampsia, suppresses invasion in EVT cell lines by inhibiting autophagy. The sENG-inhibited EVT invasion is recovered by TGF-β treatment in a dose-dependent manner. A low dose of sENG also inhibits the replacement of HUVECs by EVT cell lines in the *in vitro* model of vascular remodeling. This is the first report to show the role of autophagy in poor vascular remodeling during preeclampsia. Several studies have linked TGF-β to inhibition of EVT invasion [54–56]. Conversely, one study reported that TGF-

β augments EVT invasion [57]. It is well known that sENG binds TGF-β, thus neutralizing its effects. Paradoxically, our data showed that TGF-β neutralized the effect of sENG, resulting in recovery of HTR-8/SVneo cell invasion under hypoxic conditions (2% oxygen tension) mimicking the physiological hypoxia during the early pregnancy period, but TGF-β showed no effect on HTR-8/SVneo cell invasion under 20% oxygen tension. Thus, physiological hypoxia, in which EVT cells invade into maternal side, was the key to understand the mechanism by which sENG inhibits autophagy in EVT cells. Though TGF-β has been shown to induce autophagy accompanied with transcriptional increase of BECN1, Atg5, and Atg7 mRNA in human hepatoma cells, and increases autophagy in mammary carcinoma cells [58, 59], neutralizing effects to sENG by TGF-β might be more important than autophagy activation in EVT cells.

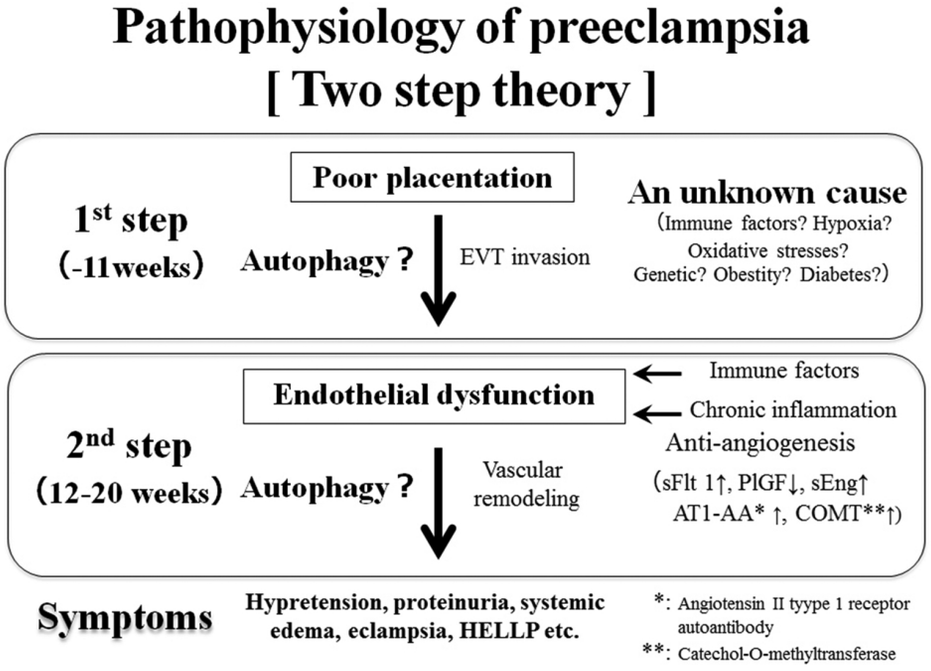

Figure 3. Two-step model of preeclampsia: Poor placentation is happened before 11 weeks of gestation, and endothelial dysfunction is happened after 12 weeks of gestation. Autophagy might be involved in each step of the pathophysiology of preeclampsia.

In regard to the role of autophagy in placenta, it remains to be elucidated whether autophagy is activated or inhibited in preeclamptic placenta. Increased numbers of LC3B dots, a marker of autophagy activation, in villous trophoblasts were observed in cases of preeclampsia with IUGR or idiopathic IUGR placentas, compared with normal human pregnancy [60]. Furthermore, sphingolipids may be involved in autophagic activation in trophoblasts in preeclamptic placentas [61]. These support the activation of autophagy in villous trophoblasts during preeclampsia and IUGR. On the other hand, p62/SQSTM1, a protein specifically digested by autophagy, accumulated in autophagy-suppressed human cell lines, suggesting inhibition of autophagy. The expression of p62/SQSTM1 is significantly higher in EVTs in preeclamptic placentas, demonstrating the inhibition of autophagy in EVTs during preeclampsia. However,

p62/SQSTM1expression in syncytiotrophoblasts is not observed in either preeclamptic placentas or normal placentas, indicating the activation of autophagy in syncytiotrophoblasts [35]. Together, these results suggest that autophagy inhibition occurs specifically in EVTs, and that there appears to be a difference in autophagic activity between syncytiotrophoblasts and EVTs in preeclamptic placentas. Sera from preeclamptic patients induce hypertension, proteinuria, and FGR in pregnant IL-$10^{-/-}$ mice, indicating that a variety of factors, including sENG and soluble Flt-1, contribute to the occurrence of preeclampsia [39]. Additionally, rapamycin-induced autophagy in peripheral blood mononuclear cells is suppressed in the presence of sera from women with preeclampsia, but not women with normotension [62]. Thus, sera from women with preeclampsia modulate the status of autophagy in the placenta.

BECN1 acts as an initiator of autophagy in mammals, and upregulation of BECN1 expression represses cellular proliferation under hypoxia. The expression of BECN1 mRNA or protein is significantly higher in IUGR without preeclampsia, than in normal pregnancy [60], and no significant difference in BECN1 expression is reported between syncytiotrophoblasts of preeclampsia or normal pregnancy patients [63]. A recent report demonstrated the importance of BECN1 for maintaining pregnancy in mice. Pregnant dams lacking BECN1 in the ovarian granulosa cell population showed impaired progesterone production during preterm labor. Luteal cells in this mouse model exhibit p62 accumulation, which indicates deficiency of autophagy, and a failure of neutral lipid storage, which is needed for steroidogenesis [14]. Progesterone in humans is produced in syncytiotrophoblasts of the placenta after 8 weeks of gestation. Despite the difference between mouse and humans, a BECN1 deficiency can be able to affect preterm birth in humans by different ways. This is the case in the hormonally-induced preterm labor model, while inflammation-induced preterm labor is also enhanced by alternation of autophagy flux, resulting in NF-kB mediated hyperinflammation in the placenta [64]. Finally, an unexpected function of autophagy in the placenta has been revealed: micro RNAs delivered from human placental trophoblasts to nonplacental recipient cells confer resistance to infection with different types of viruses, such as human cytomegalovirus, herpes simplex virus-1, vaccinia virus, poliovirus, or coxsackievirus B3. This involves exosome-mediated transfer of a unique set of placental-specific effector micro RNAs, indicating that the placenta is involved in regulating systemic immunity in humans [65].

5. Attention for estimating autophagy

Mizushima et al. [66] stated that there is no single "gold standard" for methods to monitor or modulate autophagic activity or flux. Rather, one should consider the use of several different concurrent methods (with nonoverlapping limitations) to accurately assess the status and functions of autophagic activity in any given biological setting. Of note, a common misconception for estimating autophagic activity often occurs in human tissue samples. Increased numbers of autophagosomes, LC3 dots, in cells does not invariably correspond to increased cellular autophagic activity. Tissue samples reflect only one time point when they are fixed, but "autophagy flux" could have occurred before fixation. Thus, autophagosome accumulation may represent either autophagy induction, or alternatively, suppression of steps in the

autophagy pathway downstream of autophagosome formation. The blockade of any step "downstream" of autophagosome formation increases the number of autophagosomes. In contrast, the blockade of any step "upstream" of autophagosome formation decreases the numbers of all autophagic structures. Therefore, simply determining the quantity of autophagosomes is insufficient for an overall estimation of autophagic activity. Indeed, in tissue samples, the quantities not only of autophagosomes, but also of autolysosomes, are available for estimating autophagic activity. In addition, we found that p62/SQSTM1, which becomes incorporated into the completed autophagosome and is degraded in autolysosomes, was accumulated in some trophoblast cell lines in which autophagy is suppressed by $Atg4B^{C74A}$ mutant [35]. The accumulation of p62/SQSTM1 could assist in the estimation of the level of impairment of autophagy in the placenta. Autophagy researchers anticipate that better assays will be developed to monitor autophagy, and that more specific agents will be developed to modulate autophagy. Indeed, more advances are necessary to accurately assess the status of autophagy in human tissues in order to improve clinical therapies involving modulation of autophagy.

6. Future directions

A growing body of evidence indicates that autophagy plays a key role in placentation and contributes to differences observed between normal pregnancy, preeclampsia, and IUGR. The placenta contributes to systemic immunity by activating autophagy in extraplacental cells. We believe that autophagy affects numerous functions, including protection from stress, energy regulation, immune regulation, differentiation, proliferation, and cell death in the placenta. Better molecular characterization of the autophagic pathways, as well as the possibility of genetically manipulating these cellular processes, will further elucidate the link between autophagic abnormalities and disease. The first and most important issue is how we determine the status of autophagy in the placenta. The status of a preeclamptic placenta is affected by severity, infarction, infection, degree of cell damage, genetic background, immunological status, and age. As mentioned previously, the elevation of autophagosomes observed in many disease conditions, initially interpreted as an increase in macroautophagy, is now more cautiously interpreted, because blockage of further downstream in this pathway can also produce a similar morphological signature. Thus, conditions initially labeled as having 'too much autophagy' are being currently reinterpreted as having 'a blockage in autophagic clearance'. Precise estimation of autophagy status will likely help to elucidate autophagic mechanisms implicated in placental disorders.

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