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Review: The role of autophagy in extravillous trophoblast function under hypoxia

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ABSTRACT

Autophagy, a process for cellular cleaning through the removal of intracellular components in lysosomes, is a well conserved mechanism from yeast to mammalian cells, and also contributes to the maintenance of cellular homeostasis and of the energetic balance, in cellular and tissue remodeling, and cellular defense against extracellular insults and pathogens. The role of autophagy in placentation has been clarified. Autophagy is induced in trophoblasts under physiological hypoxia during early pregnancy and seems to have a role in placentation. Recent findings suggest that impaired autophagy might induce poor placentation in preeclamptic cases. In this review, we discuss the role of autophagy and summarize the role of autophagy-related genes in placentas.

1. Introduction

Although autophagy was first observed in the early 1960s, most of the progress made in understanding it at the molecular level has occurred over the past decade. A major breakthrough has been the identification of the components of the autophagic machinery [1]. Autophagy is known to be important for maintaining cellular homeostasis as well as type II programmed cell death, which is characterized by the appearance of abundant autophagic vacuoles in the cytoplasm, and enlargement of the endoplasmic reticulum and Golgi apparatus [2]. Autophagy gives cells a bilateral character dependent on the cell type under stress. In pregnancy, the placenta is physiologically hypoxic and low in nutrients early on [3]. Therefore, for trophoblasts to function in early pregnancy, they require mechanisms to adjust to such stress. Any disruption of these mechanisms may contribute to placental dysfunction, resulting in obstetrical complications such as preeclampsia and intrauterine growth restriction (IUGR). In this review, we will focus on autophagy as a cellular cytoprotective mechanism, especially in mammalian trophoblasts.

2. What is autophagy?

Life can only be established based on a homeostatic balance between synthesis and degradation. For turnover of cellular components, eukaryotic cells are equipped with several degradation systems, one of which is the process of autophagy. Autophagy is a transport pathway leading from the cytoplasm to lysosomes. While the proteasome generally serves to selectively degrade short-lived proteins, most long-lived proteins, which constitute the majority of cellular materials, are digested in lysosomes. There are several classifications of autophagy, including macroautophagy, microautophagy and highly specialized forms of autophagy (e.g. pexophagy and mitophagy, i.e. autophagy of peroxisomes and mitochondria). These processes are distinct morphologically but, in principle, share biochemical and trafficking pathways and represent more or less bulk degradative processes. In addition to these types of autophagy, which are for the most part related, individual cytosolic proteins can be lysosomally degraded through direct import across the lysosomal membrane, in a process termed chaperone-mediated autophagy. Macroautophagy is thought to play a major role in intracellular degradation. In this review, we use the term autophagy as a synonym for macroautophagy.

During the autophagic process, a single-membrane structure, the so-called isolation membrane, surrounds portions of the cytoplasm and organelles [4] (Fig. 1). Fusion of the tips of the isolation membrane produces a spherical double-membrane autophagosome about 1 μm in diameter. Then, the autophagosome fuses with lysosomes and the sequestered contents and the inner membranes are degraded by lysosomal hydrolases. Autophagosomes have a short lifespan in contrast to other organelles. Amino acids produced by the degradation of cytosolic components can be reused by the cell; therefore, autophagy can be considered to be an efficient recycling system. In most cells (but not all; there are exceptions), autophagy is usually suppressed to a basal level. Some conditions, including starvation, hypoxia, oxidative stress, pathogen infection and hormonal

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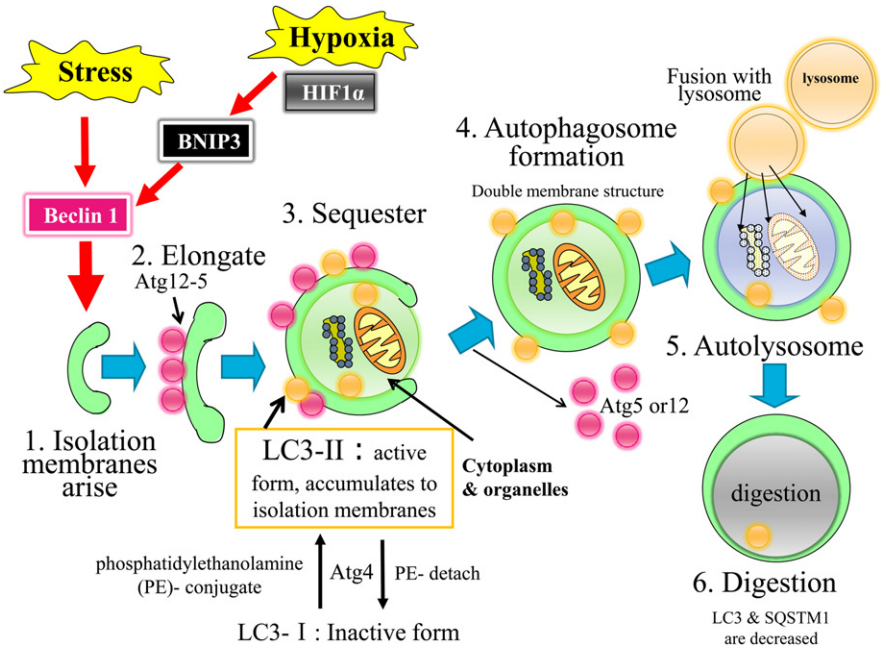

Fig. 1. The process of autophagy in mammalian cells (see the text).

stimulation, can trigger a dramatic enhancement of autophagy. Regulation of autophagy is dependent on the type of stimulation and mediated by many factors, as reviewed by Bildirici et al. [5]. Our own work has uncovered parvovirus B19 infection-induced mitophagy in erythrocytes [6].

Autophagy and autophagy-related genes have been implicated in a broad spectrum of human health issues including Alzheimer's disease, Huntington's disease, Parkinson's disease, diabetes, aging, muscle atrophy, and myopathies, with additional roles in neural stem cells in adult brain, liver, antioxidant response, lipid metabolism, and cancer. Autophagy has been associated with inflammatory illnesses such as Crohn's disease.

Cells exhibit low levels of constitutive autophagy even under normal dietary conditions. What is the role of this basal autophagy? Two independent studies using conditional knockout mice whose brains lacked Atg5 and Atg7, respectively, demonstrated that absence of this basal autophagy in brain tissue caused neurodegenerative disease. Although these mice are not genetically prone to the disease, cytoplasmic inclusion bodies accumulated in their neurons. Thus, basal autophagy is probably critical for clearance of spontaneously and constitutively generated misfolded proteins. Furthermore, accumulating evidence strongly suggests that both autophagy and the proteasome system defend neurons against aggregate-prone toxic mutant proteins (such as expanded polyglutamine-containing proteins) that cause neurodegenerative disease.

3. Placentation under hypoxic conditions

The current hypothesis regarding the etiology of preeclampsia is focused on shallow trophoblast invasion and poor placentation. Trophoblast stem cells differentiate into two cell types, villous trophoblasts and extravillous trophoblasts (EVTs), in humans. Invading trophoblasts called interstitial EVTs migrate into the decidualized endometrium and endovascular EVTs migrate along the lumina of spiral arterioles. The invasion by EVTs of spiral arteries 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 placenta in a low-oxygen environment in the first stage of pregnancy (Fig. 2). EVTs invade the maternal decidua under harsh conditions, such as low oxygen (2–5% O_2) and low glucose concentrations (1 mM), until 11 weeks of gestation [7,8]. As the EVTs then proceed into the uterus, 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. This remodeling of the spiral arteries is essential to allow a proper placental perfusion to sustain fetal growth.

The signaling pathway responsible for triggering autophagy seems to differ depending on the cell type. For example, enhanced mitochondrial autophagy (mitophagy) during hypoxia is suggested to be an adaptive response, reducing the levels of reactive oxygen species (ROS) and protecting cell integrity, although in several glioma and breast cancer cell lines, prolonged hypoxia mediates autophagic cell death. The most draconian response to persistent hypoxia is the active destruction of mitochondria by selective mitochondrial autophagy. Remarkably, mouse embryo fibroblasts (MEFs) cultured at 1% O_2 reduce their mitochondrial mass by ~75% within 48 h through autophagy that is initiated by the HIF1-dependent expression of BNIP3 (BCL2/adenovirus E1B 19 kDa interacting protein 3), a mitochondrial protein that competes with beclin1 for binding to Bcl2 (B-cell CLL/lymphoma 2), thereby freeing beclin1 to trigger autophagy. The adaptive significance of these metabolic responses to hypoxia was revealed by the finding that HIF1α-deficient MEFs died when cultured under hypoxic conditions for 72 h due to dramatically increased ROS levels. The cells could be rescued by overexpression of BNIP3 or PDK1 (pyruvate dehydrogenase kinase, isozyme 1), or by treatment with free-radical scavengers. It has long been recognized that mitochondrial ROS production increases under hyperoxic conditions. However, recent studies have demonstrated that acute hypoxia also leads to increased mitochondrial ROS production, which is required for the inhibition of HIF1α hydroxylase activity. Exposure of wild-type (WT) MEFs to hypoxia for 48 h resulted in reduced ROS levels, in contrast to HIF1$\alpha^{-/-}$ MEFs in which ROS levels were markedly