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Scholarly Dialog

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Role of autophagy in experimental varicocele: a Review

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Abstract

Autophagy is a highly conserved eukaryotic cell recycling process. Autophagy plays an important role in cell survival and maintenance through the degradation of cytoplasmic organelles, proteins and macromolecules and the recycling of degradation products. Therefore it's possible that autophagy may play an important role in the pathological process of several diseases, including varicocele. Then, is useful to clarify which are the molecular mechanisms that activate and regulate the autophagic process, in response to the stressor generated in varicocele.

Key Words: Varicocele; Autophagy; Male infertility.

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Introduction

Varicocele (VC) is an abnormal venous dilatation and/or tortuosity of the pampiniform plexus in the scrotum (1). VC is one of the main factors causing male infertility and affects about 15% of all healthy male adults and about35% of patients with primary infertility (2), so it isparticularly important to clarify the pathogenetic mechanism in the course of male infertility. There is no single pathogenetic factor that alone justifies the adverse effects of varicocele on the testes. The pathogenesis of disease is complex and multifactorial, with several proposed mechanisms acting in synergy. Indeed, blood stasis negatively affects spermatogenesis by causing an increase in scrotal temperature (usually below 2°C at body temperature), hypoxic damage (stagnant hypoxia), a reflux of renal and adrenal catabolites, and last a modification of the testicular hormonal balance.

The increase in hydrostatic pressure at the renal-spermatic level leads to reflux of renal and adrenal metabolites into the internal spermatic vein, and consequently, into the testes, resulting in vasoconstriction by norepinephrine and adrenaline in the testicular and epididymal arterioles, decreases perfusion and ischemic hypoxia, and impaired of spermatogenesis(3).

In this complex pathophysiological network, oxidative stress seems to play a central role, as the increase in reactive oxygen species (ROS) and total antioxidant capacity (CT) are thought to be responsible for germ cell damage, resulting in induction of apoptosis.

Apoptosis

Apoptosis is an orderly process that occurs in a well-orchestrated sequence of events characterized by a general reduction in the volume of the cell and its nucleus, loss of cell adhesion, disintegration of chromatin into small fragments and ending with cell death and rapid elimination of dying cells by phagocytosis. Apoptosis is involved in the elimination of cells whose genome is irreparably damaged and in cells that have completed their physiological function (4).

Autophagy

Autophagy is a process conserved during evolution that plays an important role in both physiological and pathological states. It is considered a fundamental mechanism for cell survival, as it performs the main function of degrading potentially harmful cytoplasmic components, such as damaged organelles and inadequately folded proteins that no longer function. Therefore, autophagy helps to reduce the risk of toxic protein aggregates formation. This catabolic process is activated under various conditions such as oxidative stress, heat stress, endoplasmic reticulum stress, hypoxia, infections, neoplastic processes, neurodegenerative, cardiovascular and autoimmune diseases (5).

During autophagy, a double membrane derived from the smooth endoplasmic reticulum, surrounds the damaged cytoplasmic components, forming an autophagosome; the outer membrane of the autophagosome then fuses with that of a lysosome to form an autophagolysosome and the contents of which are degraded and recycled (simple molecules, free fatty acids, amino acids and nucleotides are reused as an energy source by the cell). The body left over from this process may be excreted from the cell or retained in the cytoplasm as lipofuscin granules. There are essentially four phases of autophagy, described as follows.

Induction: In mammalian cells, the induction of autophagosome formation-occours throughof ULK1.

ULK1 is a 112-kDa serine/threonine-specific protein kinase that contains an N-terminal kinase domain, a serine-proline-rich region, and an interacting C-terminal domain(6).

ULK1, can be phosphorylated by mammalian target of rapamycin complex 1 (mTORC1) which decreas its kinase activity thus deactivating the ULK1 complex and blocking autophagyor by AMP-activated protein kinase (AMPK) to which promotes autophagy (7,9).

Once activated, ULK1 phosphorylates many targets, such as BECLIN1, which is essential for the onset of autophagy.

Nucleation: ULK1 activates phosphoryl Beclin1 and Amber1 to promote the formation and activation of the Vps34 complex. In the smooth endoplasmic reticulum, Vps34 catalyzes the conversion of phosphatidylinositol (PI) to PI3 phosphate and recruits the specific autophagy proteins necessary for phagophore formation.

Beclin1 contains three identified structural domains, including a BH3 domain, a helical central domain (CCD) and an evolutionarily conserved domain (ECD). In addition, Beclin1 regulates autophagy through several steps by associating with other specific proteins, including Ambra1 (8).

Ambra1, a cofactor of Beclin1, has also been shown to be an inseparable part of the central Vps34 complex and a positive regulator of autophagy (9).

Elongation: there are two conjugation systems involving ubiquitin-like proteins (UBLs) that contribute to phagophore elongation (10).

The first system involves the formation of the ATG12-ATG5-ATG16L1 complex (they function as in yeast). ATG12 is irreversibly conjugated to ATG5 and dependent on the activating enzyme E1 (ATG7) and the conjugating enzyme E2 (ATG10). The ATG12-ATG5 conjugate binds ATG16L1 through ATG5. ATG16L1 dimerizes and allows association with the phagophore, which promotes membrane expansion (11).

The second UBL system involved in phagophore expansion is the LC3/ATG4 system.

The form of LC3 processed with ATG4 is referred to as LC3-I and the form conjugated with the lipid phosphatidyl-ethanolamine (PE) is referred to as LC3-II.

LC3 is processed by ATG4 to recognize a C-terminal glycine (LC3-I). ATG7 activates LC3-I and transfers it to the E2-like enzyme (ATG3). The ATG12-ATG5-ATG16L1 complex can participate as an E3 ligase in the conjugation of PE to LC3-I to form LC3-II, which can bind to the phagophore. LC3-II can subsequently be cleaved from ATG4 to release LC3 and PE (deconjugation).

Another protein thought to be involved in phagophore elongation is the transmembrane protein ATG9. It appears to be necessary for the autophagosome membrane recruitment; undernutrient-rich conditions, ATG9 is localized in the trans-Golgi network and in endosomes. When cells are starved of nutrients, ATG9 accumulates along with autophagosomal markers. This switch to autophagosomes depends on both ULK1 and PtdIns3K activity and is negatively regulated by MAPK14/p38a(10).

Autophagosome completion and fusion: phagophore must eventually mature to form a complete autophagosome, which fuses with an endosome or lysosome and becomes an autophagolysome.The movement of autophagosomes to lysosomes is independent of microtubules. The VTIIB protein in involved in the fusion of autophagosome with endosomes. During UVRAG, it can bind to the PtdIns3K complex, activate the GTPase RAB7, and promote fusion with lysosomes (10).

Role of autophagy in experimental varicocele

Thermal stress

Testicular heating is known to suppress spermatogenesis in several mammalian species, including humans. When the testis is exposed to body temperature or higher, as is the case with varicocele, there is increased degeneration, death, and loss of germ cells through the apoptotic pathways.

The results of the study [Zhang, Jiang, Bi, Zhu, Zhou, Sha](12) showed that testicular heating is responsible not only for the activation apoptosis but also autophagy in germ cells. In particular, this study found that hyperthermia determines several specific features of the autophagy process, including the formation of autophagosomes and the conversion of LC3-I to LC3-II.

Oxidative stress

In response to hypoxia mammalian cells activate hypoxia-inducible factors (HIFs). HIFs play a crucial role in regulating the transcription of more than 100 genes involved in homeostasis such as glycolysis metabolism, biosynthesis, angiogenesis, cell survival (hence autophagy), cell proliferation and growth, but also apoptosis (13).

HIF consists of one of three α subunits (1, 2, and 3) and one β-subunit (14). HIF-β is constitutively expressed, where HIF-α is induced by hypoxia. HIF-2α shows 48% amino acid sequence homology with HIF-1α, HIF-1α, although and HIF-2α have different specificity in their transcriptional targets: HIF-2α is most abundantly expressed at the embryonic development stage and in adult vascular endothelial cells, lungs, placenta and heart. HIF-1α, on the other hand, is ubiquitously expressed in all mammalian tissues and cell types, mainly in the cytoplasm of spermatogenic cells and in the vascular endothelium of the testicle(15).

Numerous studies have documented that testicular hypoxia in varicocele positively affects HIF-1α expression, and how HIF-1α is significantly increased in the testis of varicocele rats compared to the control group(16,3,17,18). Other studies have shown that the level of HIF-1α protein in the internal spermatic vein (ISV) of patients with varicocele was also significantly higher in males than in healthy subjects (19,20).

HIF-1α is associated with oxidative stress and both regulate the production of ROS(21,22). Under hypoxic conditions, mitochondria can produce a large amount of ROS, which activates HIF-1α signaling and thus increase ROS(23). On the other hand, increased ROS levels lead to increased expression of p53 in varicocele rats under hypoxia conditions (24).

Hypoxia and apoptosis: One of the main regulators of apoptosis is p53(25),which transcriptionally targets the Bax gene. In severe hypoxic stress, the expression of both factors, p53 and HIF1-α, is increased, predisposing cells to apoptosis and thus cell death(26).

Another study showed that the expression of HIF-1α, Bax and caspase-3 increased in varicocele rats compared to the control group, while Bcl-2 was reduced(27).

Hypoxia and autophagy: The study [Zhu and Rao](28) also showed that an important role of hypoxia is to induce HIF-1α, which is responsible for activation of a number of autophagy-related genes, such as BNIP3. The BNIP3 protein (whit BH3 domains), interacts with Bcl-2 or Bcl-XL, forming a heterodimer, and releasing Beclin1, from the Beclin-1/Bcl-2 complex (under physiological conditions, Beclin-1 and Bcl-2 inhibit the activation of autophagy), allowing Beclin-1 to activate autophagy pathway. Next, Beclin1 combines with Vps34 and UVRAG to form phosphoinositide 3-kinase class III and then forms phosphatidylinositol 3-phosphate (PIP3P), which is critical for the autophagy process. Consequently, the HIF-1α/BNIP3/Beclin1 pathway is an important way to induce autophagy under hypoxic conditions.

Conclusions

Overall, these data from experimental research in the literature have previously shown that in the rat varicocele model, heat stress, hypoxia and oxidative stress are responsible not only for activation of the apoptotic process but also of autophagy. The present study has made it possible to understand which of the myriad proteins are involved in autophagy. Further studies in humans, which we are interested, are needed to clarify the role in clinical varicocele and to open new scenarios for the development of pharmacological treatments that can support surgical therapy and thus be able to treat or limit the damage caused by the germ cells of men with varicocele.

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