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INVITED RESEARCH HIGHLIGHT

Sperm Biology

The impact of autophagy in spermiogenesis

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Autophagy is an evolutionarily conserved self-digestion process which is essential to keep basal homeostasis in a cell. During this process, degradation and recycling of many cytoplasmic components including the long-lived, unnecessary or aggregated proteins and damaged organelles is achieved through lysosomal machinery. Autophagy has a critical role for lower eukaryotic organisms such as yeast to survive and adapt to nutrient starvation conditions. In addition to this primary function, autophagy appears as a crucial mechanism for cell differentiation and development enabling the cells to modify their content and morphology in response to environmental and hormonal cues. A recent study by Shang *et al.*[1] shed more light on the molecular mechanisms of how autophagy regulates spermiogenesis.

Three types of autophagy are described in higher eukaryotes, namely, macroautophagy, microautophagy, and chaperone-mediated autophagy; however, macroautophagy (hereafter referred to as autophagy) appears to be the main pathway.[2] Autophagy starts with an isolation membrane which elongates and engulfs cytoplasmic cargo leading to the formation of a double-membrane vesicle, known as the autophagosome. Autophagosomes move along cytoskeleton and mature through fusion with the lysosome, where the degradation of autophagosomal contents, including the autophagosome membrane, takes place by lysosomal hydrolases. The by-products of the degradation, such as amino acids, are then exported back to the cytoplasm to serve as building blocks for the newly synthesized macromolecules. Our understanding of the core molecular mechanisms of autophagy comes from the studies in yeast by the discovery of autophagy-related (ATG) genes, many of which also have known homologs in other eukaryotes. Two ubiquitin-like conjugation systems, the ATG12 and microtubule-associated protein light chain 3 (LC3; mammalian homolog of ATG8) systems, are key to autophagy.[3] A ubiquitin-activating E1-like enzyme, ATG7, is required for the activation of both ATG12 and LC3B in an ATP-dependent manner. After being activated by ATG7, ATG12 is transferred to the E2-like enzyme ATG10, and eventually conjugated to ATG5. Finally, ATG12-ATG5 conjugate forms a complex with ATG16L1 (mammalian homolog of ATG16). LC3B contains an additional sequence at its C-terminus, which has to be removed by ATG4, a cysteine protease, to generate LC3-I before it can be activated by ATG7 and ATG3 (E2-like enzyme), sequentially. ATG12–5–16L1 complex acts as an E3 ubiquitin ligase for this second ubiquitin-like conjugation system, resulting in conjugation of LC3-I to lipid phosphatidylethanolamine (PE), finally generating LC3-II. LC3-II is found on both the internal and external surfaces of the autophagosome and is important for membrane expansion and closure, and in selecting cargo for degradation. ATG4 can cleave the external LC3 from the autophagosome for reuse, and this reaction can also regulate autophagosome formation.

Spermiogenesis is the final stage of spermatogenesis, which transforms round spermatids into mature spermatozoa.[4] During spermiogenesis, round spermatid undergoes a series of complex and significant structural and biochemical changes: (1) Nuclear chromatin condensation occurs due to the replacement of histones by protamines. (2) A cap-like membrane-bound organelle, called acrosome, is formed through coalescence of the coated vesicles budding from the trans-Golgi network and is located over the anterior part of the spermatid nucleus. The acrosome is a lysosome-related organelle which carries hydrolytic enzymes to facilitate sperm penetration through the zona pellucida. An F-actin, myosin and keratin- containing cytoskeletal plate named acroplaxome is assembled in the subacrosomal space of mammalian spermatids, which anchors the developing acrosome to the nuclear envelope during shaping of the spermatid head.[4,5] While the spermatid nucleus is elongating and the acrosome is forming, the transient microtubule- and actin-containing manchette develops caudally to the acrosome. The acrosome-acroplaxome-manchette complex is one of the major drivers for the shaping of the sperm head. (3) During the elongation phase of spermiogenesis, in round spermatid nucleus, a pair of centrioles move to the opposite pole of where the acrosome begins to form and the axoneme starts to develop from the distal centriole gradually extending out into the cytoplasm. Axoneme is the central component of the flagellum and consists of a central pair of microtubules surrounded by 9 outer doublet microtubules (the so-called "9 × 2 + 2" arrangement). (4) Another major event during spermiogenesis is the transportation of the cytoplasm toward the sperm tail along the manchette and finally its removal. A failure in the cytoplasmic removal results in defects in sperm head shaping and also affects the motility of the sperm.

There is evidence that autophagy is involved in spermatogenesis.[1,6–12] It was shown that heat stress can induce autophagy in addition to apoptosis in mouse germ cells[9] and autophagosomes were detected in cultures of primary rat spermatocytes.[11] Another study showed that *Ol-epg5* (ectopic P-granules autophagy protein 5 homolog)-knockout (KO) in medaka fish results in an impaired spermatogenesis and germplasm clearance.[12]

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These data suggest that autophagy might be crucial for spermatogenesis. However, the functional roles of autophagy and their underlying molecular mechanisms in spermatogenesis are still largely unknown. Using germ cell-specific *Atg7*-KO mice, Shang *et al*.[1] showed that autophagy regulates the cytoplasmic remodeling during spermatid differentiation, particularly the F-actin network. They found a severe reduction in the motility of the spermatozoa with morphological defects, such as bent head, coiled tail, and aggregation, which are caused by the inability to remodel the actin cytoskeleton and disorganization of the "9 × 2 + 2" structure of the flagellum. Previously, the same group had also demonstrated that *Atg7*-KO mice have a defect in acrosome biogenesis with irregular or nearly round-headed spermatozoa, which is similar to human globozoospermia.[6] The study of Shang *et al*.[1] in combination with the study of Zhuo *et al*.[13] led to the identification of PDLIM1 protein as a negative regulator of cytoskeleton organization in spermatids which has to be degraded by autophagy to enable the correct assembly of the actin- and microtubule-containing structures that have crucial roles in regulating many steps during spermiogenesis. The effect of the cytoskeleton on autophagy is well known; however, the current study[1] has prompted us to look at the relationship between cytoskeleton organization and autophagy in a different perspective. Until now, few studies focused on how autophagy modulates cytoskeletal organization.[7] Shang *et al*.[1] provided more evidence about the mutual modulation of autophagy and the cytoskeleton organization by each other, and this mutual modulation might drive the molecular events taking place during many differentiation processes. Interestingly, a study[7] by the same group has shown that PDLIM1 also accumulates in autophagy-deficient Sertoli cells leading to subfertility due to the disorganized seminiferous tubules and spermatozoa with aberrant head morphology. They found that PDLIM1 accumulation especially led to the disruption of so called apical ectoplasmic specializations (a stack of F-actin-containing hoops that embrace the apical region of the elongating spermatid nucleus), which are suggested to have a role in sperm head shaping.

Given the importance of actin-containing cytoskeletal structures in most of the events that occur during spermiogenesis, it is important that the proteins necessary for the formation of these structures are tightly regulated. The study by Zhuo *et al*.[13] revealed more proteins other than PDLIM1 that might have negative effects on cytoskeleton remodeling. Future studies on these proteins might expand our knowledge on how they might impact differentiation processes of different cell types. Another future direction could be to check whether the defects in F-actin networks due to the lack of autophagy might also impact chromatin compaction process since it has been suggested that acrosome development and chromatin remodeling are interacting processes.[14] Autophagy-deficient mice could be an interesting model to study whether there is a crosstalk between these two important molecular events that take place at the same time.

COMPETING INTERESTS

All authors declared no competing interests.

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