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Autophagy-disrupted LC3 abundance leads to death of supporting cells of human oocytes

Woojin Kang[a,b,1], Eri Ishida[a,1], Kenji Yamatoya[c,1], Akihiro Nakamura[b,d], Mami Miyado[e], Yoshitaka Miyamoto[b], Maki Iwai[b,d], Kuniko Tatsumi[a], Takakazu Saito[a], Kazuki Saito[a], Natsuko Kawano[f], Toshio Hamatani[d], Akihiro Umezawa[b], Kenji Miyado[b,*], Hidekazu Saito[a,*]

[a] Department of Perinatal Medicine and Maternal Care, National Center for Child Health and Development, 2-10-1 Okura, Setagaya, Tokyo 157-8535, Japan
[b] Department of Reproductive Biology, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya, Tokyo 157-8535, Japan
[c] Department of Applied Biological Science, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan
[d] Department of Obstetrics and Gynecology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan
[e] Department of Molecular Endocrinology, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya, Tokyo 157-8535, Japan
[f] Department of Life Sciences, School of Agriculture, Meiji University, 1-1-1 Higashimita, Kawasaki, Kanagawa 214-8571, Japan

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ABSTRACT

Autophagic recycling of cell parts is generally termed as the opposite of cell death. Here, we explored the relation between cell death and autophagy by examining granulosa cell layers that control oocyte quality, which is important for the success of fertilization. Granulosa cell layers were collected from infertile women and morphologically divided into four types, viz., mature (MCCs), immature (ICCs), and dysmature cumulus cells (DCCs), and mural granulosa cells (MGCs). Microtubule-associated protein light chain 3 (LC3), which is involved in autophagosome formation, was expressed excessively in DCCs and MGCs, and their chromosomal DNA was highly fragmented. However, autophagy initiation was limited to MGCs, as indicated by the expression of membrane-bound LC3-II and autophagy-related protein 7 (ATG7), an enzyme that converts LC3-I to LC3-II. Although pro-LC3 was accumulated, autophagy was disabled in DCCs, resulting in cell death. Our results suggest the possibility that autophagy-independent accumulation of pro-LC3 proteins leads to the death of human granulosa cells surrounding the oocytes and presumably reduces oocyte quality and female fertility.

1. Introduction

In mammals, ovarian follicles consist of three types of layered somatic cells, viz., theca, granulosa, and cumulus cells (CCs), in addition to oocytes [1,2]. When each ovarian follicle matures, CCs differentiate from multiple layers of granulosa cells and surround a single oocyte. Despite their morphologically similarity, CCs play a critical role in the formation of fertilization-competent oocytes.

Macroautophagy (hereafter autophagy) often occurs prior to apoptosis [3]. Membrane vesicles, termed as autophagosomes, enclose a part of the cytoplasm and organelles, and its contents are degraded by the unification of lysosome and autophagosome (Fig. 1A). Autophagy governs both apoptosis and clearance of cellular waste, thereby maintaining cellular homeostasis. LC3, a mammalian homologue of yeast Atg8 (Aut7/Apg8), localizes to the autophagosomal membranes after posttranslational modifications [4]. Autophagy-related genes (Atgs) participate in autophagosome formation, and LC3 is a convincing marker of initiation of autophagosome formation. The C-terminus of LC3 is cleaved by Atg4 protease, which generates cytosolic LC3-I; LC3-I is conjugated to phosphatidylethanolamine (PE) and lipidated in a ubiquitin-like reaction that requires Atg7, one of the E1-like enzymes. This lipidated product, LC3-II, is bound to the membrane of autophagosomes. Finally, aging cells and damaged organelles are degraded by the fusion of autophagosomes with lysosomes [5].

Autophagy has been investigated with respect to reproduction and early embryogenesis. Interaction between apoptosis and autophagy promotes the formation of corpus luteum and the survival of androgen-secreting cells [6]. Autophagy functions during gametogenesis because autophagy is indispensable to synthesize a new zygote protein from resolvents of the maternal proteins [7,8]. However, in fish, apoptosis occurs in the follicular atresia simultaneously with autophagy [9]. Apoptosis is also known to occur in both human cumulus and granulosa

* Corresponding authors.
E-mail addresses: miyado-k@ncchd.go.jp (K. Miyado), saitou-hi@ncchd.go.jp (H. Saito).
[1] Authors equally contributed to this work.

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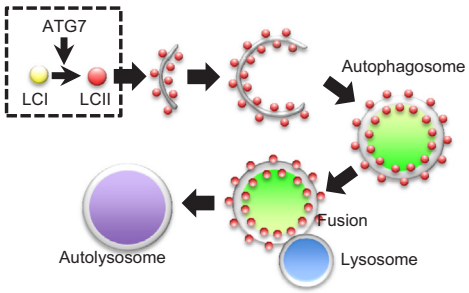
A



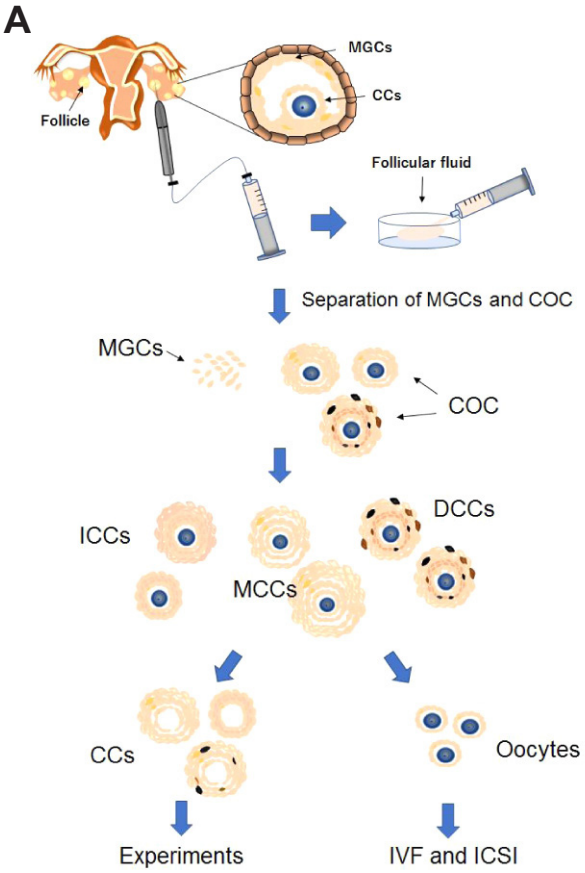
B

Number of patients	9
Age (years)	38.8 ± 1.3
Day3 FSH (mIU/ml)	4.6 ± 0.4
Day3 LH (mIU/ml)	2.8 ± 0.6
Day3 estradiol (pg/ml)	31.2 ± 4.8
Day of hCG estradiol (pg/ml)	3266.7 ± 549.2
Day of hCG progesterone (ng/ml)	0.5 ± 0.1
Day of hCG FSH (mIU/ml)	15.0 ± 1.6
Day of hCG LH (mIU/ml)	2.1 ± 0.4
Ampoules of gonadotropin used*	21.4 ± 1.7
Days of stimulation	13.0 ± 0.5
Number of follicles aspirated	14.2 ± 1.9
Number of oocytes retrieved	12.6 ± 1.5
Number of mature COCs**	6.2 ± 1.2
Number of immature COCs	4.1 ± 0.6
Number of dysmature COCs	2.2 ± 0.4
Number of embryos fertilized	6.6 ± 1.5

*1 ampoule = 75IU
**COCs: Cumulus-oocyte-complex

Fig. 1. General scheme of autophagy and clinical data of the patients. **A**, Scheme of autophagy and participation of ATG7 in LC3 activation during autophagy. **B**, Clinical demographic data of the patients (mean ± SE).

A



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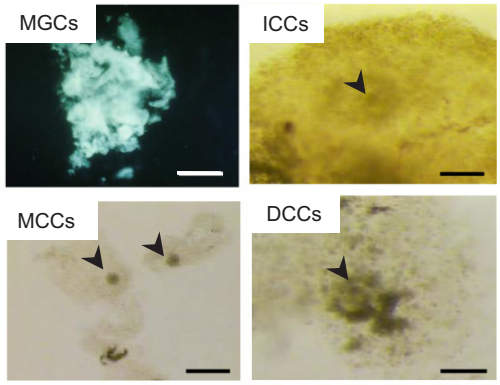

C

	Total number of oocyte	Number of fertilized oocytes	Fertilized oocyte (%)
ICCs	837	450	53.8
MCCs	1359	825	60.7
DCCs	321	109	34.0*

* Significant difference among groups
(ICCs v.s. DCCs, $p<0.001$; MCCs v.s. DCCs, $p<0.001$)

Fig. 2. Flow chart for collecting samples and fertilization rate by maturity of cumulus cells. **A**, Flow chart of collecting cumulus cells (CCs) from cumulus-oocyte complex (COC). COCs were retrieved from stimulated follicle by hormones. Human granulosa cells (MGCs) and COCs were isolated from follicular aspirates. The maturity of COCs was evaluated based on morphologic criteria. Evaluated CCs were physically detached from COCs. Obtained CCs were used in experiments and oocytes were used for ART by IVF or ICSI. **B**, Classification of MGCs and CCs. The cells were divided into four cell types, viz., MGCs, ICCs, MCCs, and DCCs. Arrowheads indicate oocytes. Scale bars, 100 μm. **C**, Fertilization rate of oocytes separated from ICCs, MCCs, and DCCs. Table shows general fertilizatuion rate using oocytes that were separated based on maturity of CCs.

cells; however, its connection with autophagy in mammalian reproduction remains unknown. In this study, we explored the role of autophagy in maintaining CC quality.

2. Materials and methods

2.1. Patients and follicle stimulation protocol

Human granulosa cells [cumulus and mural granulosa cells (MGCs)] were obtained from patients treated with assisted reproductive