Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta Morphologica et Anthropologica, 29 (3-4)
Sofia ● 2022

Preliminary Observations on Apoptotic Fragmentation of Cultured Mouse Oocytes

Maya Markova[1], Anton Kolarov[1], Irina Chakarova[1], Valentina Hadzhinesheva[1], Ralitsa Zhivkova[1], Stefka Delimitreva[1], Milena Mourdjeva[2], Venera Nikolova[1]*

[1]*Department of Biology, Medical Faculty, Medical University of Sofia, Sofia, Bulgaria*
[2]*Department of Molecular Immunology, Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria*

*Corresponding author e-mail: mayamarkov@gmail.com

We studied apoptotic fragmentation in ovulated mouse oocytes. Some cells were fixed immediately after isolation, while others were cultured for 3 hours with or without prostaglandin F2 alpha. Membrane organelles, fibrillar actin and DNA were stained with DiOC6, TRITC-phalloidin and Hoechst 33342, respectively. While fragmented cells were generally rare, most of them were in samples treated with prostaglandin F2α, revealing it as a potential inducer of apoptosis. The chromatin had interphase appearance, indicating exit from meiosis or arrest at germinal vesicle stage. Fragmentation tended to be more pronounced in the vicinity of chromatin, which could be explained with the concentration of actin in the cap region. These preliminary data confirm the active participation of cytoplasm in oocyte apoptosis, and suggest that this process could be induced by mediators of inflammation.

Key words: Oocytes, apoptosis, fragmentation, postovulatory aging, prostaglandin F2α

Introduction

The morphology of programmed cell death (apoptosis) is characterized by fragmentation of the cell [6]. So far, few studies have addressed this process in mature ovulated oocytes where it is complicated by their metaphase II arrest. After induced apoptosis in ovulated oocytes, cytoplasmic fragmentation reportedly requires activation and exit from meiosis [3]. In line with this, ovulated eggs undergoing degeneration known as postovulatory aging are often activated and exit metaphase II [7], and apoptosis of dividing somatic cells (a.k.a. mitotic catastrophe) typically includes formation of nuclear envelopes around chromosome clusters [9]. Postovulatory aging and apoptosis of oocytes can be influenced by culture conditions such as presence of signaling

molecules. The inflammation-associated signal prostaglandin F2α has been reported to induce both cell survival [1] and apoptosis [8] in different cell types, and even in the same cell type [2]. We have investigated the impact of prostaglandin F2α on ovulated mouse oocytes, and the main effect observed was degeneration of the cytoskeleton [4]. In the present study, untreated and prostaglandin F2α-treated ovulated oocytes were examined for cytoplasmic fragmentation indicating apoptotic cell death.

Materials and Methods

All experiments conformed to legislature and ethical guidelines concerning animal research. Oocytes were obtained as described in [4] and stained as in [5] with small modifications. Briefly, female ICR mice were stimulated with follicle-stimulating hormone and luteinizing hormone, 7.5 IU each. After 48 h, ovulation was induced by 10 IU of human chorionic gonadotropin, and oocytes were collected on the next day. Some of them were immediately fixed in 2% paraformaldehyde, while others were cultured for 3 hours in α-MEM medium with or without prostaglandin F2α (50 or 100 ng/ml) before fixation. Membrane organelles, fibrillar actin and DNA were visualized using the lipophilic dye DiOC6, TRITC-phalloidin and Hoechst 33342, respectively. Cells were observed by epifluorescence and confocal microscopy.

Results and Discussion

Apoptotic fragmentation was observed in a small proportion of oocytes. It was absent in freshly isolated oocytes and very rare in those cultured without prostaglandin. Though the vast majority of prostaglandin-treated cells also showed no fragmentation, most fragmented cells were prostaglandin-treated, indicating its possible pro-apoptotic action.

Fragmented oocytes had condensed interphase chromatin, indicating either exit from meiosis or arrest at germinal vesicle stage, in accordance with literature data [3]. In some cells, nucleolus-like bodies could be seen (**Fig. 1A**).

When fragmentation was limited to a part of the cell, it tended to be more pronounced in the vicinity of chromatin, which could be explained with the concentration of fibrillar actin in the cap region. In some oocytes, membrane staining was particularly strong in the zona pellucida (**Fig. 1B**). This was most likely due to membrane material released from the fragmenting cell and captured in the zona matrix. These morphological peculiarities of the process, and the role of prostaglandin F2α as its potential inducer, are not discussed in the literature available to us and so provide new details about apoptosis of ovulated mammalian oocytes.

Conclusions

Our preliminary study confirms the active participation of cytoplasm in oocyte apoptosis, and suggests that apoptotic fragmentation could be induced by mediators of inflammation such as prostaglandin F2α.

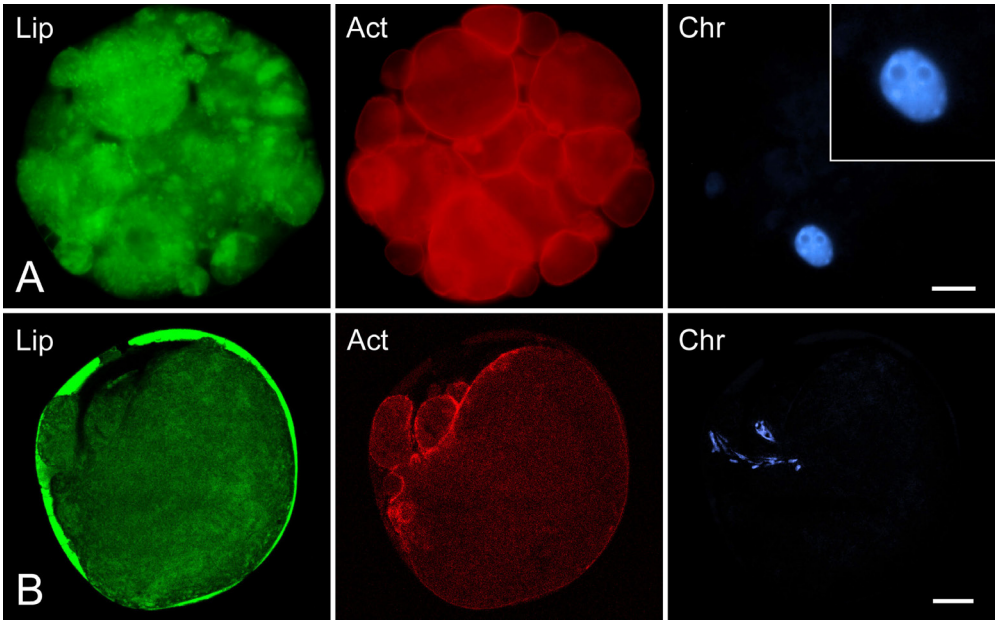

Fig. 1. Apoptotic fragmentation in mouse ovulated oocytes cultured for 3 h, staining for membranes (Lip), fibrillar actin (Act) and DNA (Chr). **A.** Complete fragmentation in a cell treated with 50 ng/ml prostaglandin F2α, epifluorescence. The main nuclear fragment, shown magnified in the inset, has two nucleolus-like bodies. **B.** Partial fragmentation in the chromatin-containing region of a cell treated with 100 ng/ml prostaglandin F2α, confocal microscopy. Zona pellucida is brightly stained for membrane material. Bars = 20 μm.

Acknowledgements: This work was supported by Grant No. D-97/24.06.2020 of the Medical University of Sofia and by the Bulgarian Ministry of Education and Science under the National Program for Research "Young Scientists and Postdoctoral Students".

References

1. **Cheng, Y., L. Peng, X. Deng, T. Li, H. Guo, C. Xu, T. Fang, X. Liu, B. Sun, L. Chen.** Prostaglandin F2α protects against pericyte apoptosis by inhibiting the PI3K/Akt/GSK3β/β-catenin signaling pathway. – *Ann. Transl. Med.*, **9**, 2021, 1021.
2. **Jonczyk, A. W., K. K. Piotrowska-Tomala, D. J. Skarzynski.** Effects of prostaglandin F2α (PGF2α) on cell-death pathways in the bovine corpus luteum (CL). – *BMC Vet. Res.*, **15**, 2019, 416.
3. **Jurisicova, A., H. J. Lee, S. G. D'Estaing, J. Tilly, G. I. Perez.** Molecular requirements for doxorubicin-mediated death in murine oocytes. – *Cell Death Differ.*, **13**, 2006, 1466-1474.
4. **Kolarov, A. I., V. P. Hadzhinesheva, I. V. Chakarova, R. S. Zhivkova, S. M. Delimitreva, M. D. Markova, M. S. Mourdjeva, V. P. Nikolova.** Prostaglandin F2α causes fast degenerative changes in ovulated mouse oocytes. – *Folia Biol. (Praha)*, **67**, 2021, 208-212.
5. **Kolarov, A., N. Mladenov, I. Chakarova, N. Ishkitiev, M. Markova, R. Zhivkova, S. Delimitreva, V. Nikolova.** Fast, easy staining method to visualize cell morphology and apoptosis. – *Acta Morphol. Anthropol.*, **27**, 2020, 3-7.

6. Perez, G. I., X. J. Tao, J. L. Tilly. Fragmentation and death (a.k.a. apoptosis) of ovulated oocytes. – *Mol. Hum. Reprod.*, **5**, 1999, 414-420.
7. Prasad, S., M. Tiwari, B. Koch, S. K. Chaube. Morphological, cellular and molecular changes during postovulatory egg aging in mammals. – *J. Biomed. Sci.*, **22**, 2015, 36.
8. Sharma, A. K., R. K. Sharma. Effect of prostaglandins E2 and F2α on granulosa cell apoptosis in goat ovarian follicles. – *Iran. J. Vet. Res.*, **21**, 2020, 97-102.
9. Vakifahmetoglu, H., M. Olsson, B. Zhivotovsky. Death through a tragedy: mitotic catastrophe. – *Cell Death Differ.*, **15**, 2008, 1153-1162.