ASYMMETRIC DISTRIBUTION OF MICROFILAMENTS, PERICENTRIOLAR MATERIAL, GOLGI APPARATUS AND MITOCHONDRIA IN OOCYTES FROM MOUSE NEONATAL OVARIA

Valentina Hadzhinesheva*, Stefka Delimitreva*, Maya Markova*, Venera Nikolova*, Irina Chakarova*, Milena Mourdjeva, Pavel Rashev**, Ralitsa Zhivkova***

** Department of Biology, Medical Faculty, Medical University of Sofia*
2, Zdrave Str.
1431 Sofia, Bulgaria
e-mail: rzhivkova@yahoo.com
*** Institute of Biology and Immunology of Reproduction,*
Bulgarian Academy of Science
73 Tzarigradsko shosse
1113, Sofia, Bulgaria

Abstract:

The breakdown of synchronously dividing cells of the ovarian cysts leads to primordial follicle formation. Meiotic division is accompanied by asymmetric distribution of some ooplasmic components and formation of Balbiani body. Our study was aimed to study the distribution of oplasmic structures involved in oocyte asymmetry during primordial follicle formation in mouse.

Methods: Ovaries of newborn mice were isolated, fixed and paraffin embedded at the first day after birth. Deparaffinized and rehydrated slides were processed for innmunohistochemistry. Immunofluorescent detection of pericentriolar material, Golgi apparatus and mitochondria was performed by antibodies. Phalloidin-TRITC was used to visualize the microfilaments. Fluorescent results were subjected to confocal laser-scanning microscopy.

Results: In oocytes, Golgi complex was detected as perinuclear circular structure and in the cytoplasmic bridges connecting oocytes. The actin was associated with the Golgi ring and showed a positive reaction at the oocyte periphery. Both Golgi apparatus and actin distribution were recognized as corresponding to Balbiani body. In oocytes, PCM and the mitochondrial marker were located near the nucleus as single or double cluster. The reaction was stronger for the mitochondrial marker. PCM1 and VDAC1 were visualized also in the cytoplasmic bridges connecting oocytes.

Conclusions: The presence of PCM1 and VDAC1 and the specific distribution of fibrillar actin suggest their mediating role in the organelle transfer through intercellular bridges during early mammalian oogenesis. The perinuclear localization of PCM1, Golgi apparatus and VDAC1 is a part of the oocyte asymmetry associated with Balbiani body formation in mammalian oocytes during primordial follicle formation.

Key words: *mammalian oocytes, Golgi complex, pericentriolar material PCM1, mitochondrial marker VDAC1, actin*

Introduction:

In oocytes of most animals, the cytoplasm is characterized by polarized asymmetric distribution of organelles and macromolecules. This polarity is created during oogenesis, may undergo final adjustment at fertilization and contributes to the morphogenesis of the early embryo (Gard , Cha and King, 1997). A structure important for the development of oocyte asymmetry is the so called Balbiani body (Kloc et al, 2004). It is a transient perinuclear aggregate of mitochondria, Golgi membranes and inclusions observed in oocytes of various metazoans during the first meiotic division (Marlow and Mullins, 2008; Zhivkova et al, 2013; Hadzhinesheva et al, 2015). It is associated with the centrosome and hence is subordinate to the primary nucleus – centrosome polarization axis of eukaryotic cells. The Balbiani body is disassembled during oocyte maturation, leaving the cytoplasm spatially organized into gradients.

The evolution of eutherian (placental) mammals has led to major changes in their oogenesis and embryonic development. As extraembryonic membranes became more and more integrated with the uterus, the telolecithal egg of the early mammalian ancestor underwent multifold secondary

reduction. As a result, oocyte cytoplasmic gradients lost their importance. However, a Balbiani body can be observed in mammalian fetal oocytes and, in species with short generation time such as mice, even in neonatal oocytes (Pepling et al, 2007). This suggests that the developmental potential of the mammalian egg may still require subtle organization of its cytoplasm. The data in this respect are scarce and require detailed studies on the structure and development of mammalian Balbiani body. To visualize and trace it, we have performed immunohistochemical localization of selected organelles and cytoskeletal components in sections of newborn mouse ovaria.

Our study was aimed to study the distribution of ooplasmic structures involved in oocyte asymmetry during primordial follicle formation in mouse.

Materials and methods:

Ovaries of BALB/c mice were isolated, fixed and paraffin embedded at the first day after birth. Five micrometer thick sections were prepared for innmunohistochemistry as follows: deparaffinized and rehydrated slides were boiled in saline-sodium citrate (SSC) buffer pH=6 for 20 min at 95^{o}C and cooled at room temperature. The unspecific antibody reaction was prevented by FITC-protein blocking (Quartett, Germany) for 15 min and Super Block (ScyTek, USA) applied for 5 min. First antibodies for Golgi complex (Golgi peripheral membrane protein p65/GRASP65, Quartett, Germany), mitochondrial marker VDAC 1 (Quartett, Germany) and pericentriolar material (PCM 1, Santa Cruz Biotechnology, USA) were applied for 1 hour and detected by FITC-labelled second antibodies. Mitochondrial marker was visualized by TRITC- conjugated second antibody and phaloidin-TRITC was used to detect the fibrilar actin. Immunofluorescent detection of pericentriolar material, Golgi apparatus and mitochondria was performed by antibodies. Phalloidin-TRITC was used to visualize the microfilaments. The chromatin was stained by Hoechst 33258 (Sigma-Aldrich, Germany) for 5 min, the slides were immersed in Polyvinyl-alcohol (Fluka, Germany). Fluorescent reaction was detected by confocal laser-scanning microscopy at 0,2μm optical sectioning (Leica TCS SPE, Leica, Germany).

Results:

Immunofluorescent detection of cytoplasmic structures showed the following asymmetric distribution within oocytes:

Golgi complex was detected as perinuclear circular structure. Actin showed association with the Golgi ring and positive reaction at the oocyte periphery. Both Golgi apparatus and actin distribution were recognized as corresponding to Balbiani body.

Pericentriolar material PCM1 and the mitochondrial marker VDAC1 were located near the nucleus as single or double cluster. Fluorescent results for both pericentriolar material and mitochondrial marker were associated with peripherally located heterochromatin of the oocyte nucleus. The reaction was stronger for the mitochondrial marker.

Microfilaments, PCM1 and VDAC1 were detected also in the cytoplasmic bridges connecting oocytes.

After observations of multiple ovarian sections, the sites of positive reaction were summarized on the following generalized schematic drawings: Fig. 1 – for Golgi material and microfilaments, and Fig. 2 – for pericentriolar material and mitochondria.

Discussion:

Asymmetric distribution of ooplasmic components is an ancient mechanism for cell fate determination in animal development that is crucial in most metazoan phyla. The Balbiani body as an aggregate of various organelles, cytoskeletal structures and inclusions simultaneously provides spatial cues for polarized distribution of numerous components (Marlow, 2010, Zhivkova et al, 2013). In oocytes of placental mammals, the Balbiani body is an evolutionary relic that could nevertheless facilitate the early stages of oogenesis before the prophase I meiotic arrest. Our results

showing perinuclear localization of PCM1, Golgi apparatus, actin and VDAC1 suggest that pericentriolar material, sorting machinery and energy production are concentrated in an asymmetric fashion in mammalian oocytes during primordial follicle formation.

The tissue sections used for the current study corresponded to the moment when oocytes were approaching their prophase I meiotic arrest, ovarian cysts were breaking down and primordial follicles were formed (Bristol-Gould et al, 2006). In this period, oocytes are still connected by intercellular bridges to ensure synchronous coordinated differentiations (Pepling, 1998). The presence of PCM1 and VDAC1 and the specific localization of fibrillar actin in sites of intercellular contacts suggest their mediating role in the organelle transfer through the bridges during early mammalian oogenesis.

In conclusion, the presented immunohistochemical study provided data for involvement of Golgi apparatus, fibrillar actin and mitochondria in the Balbiani body formation and in the maintenance and function of the intercellular bridges. Based on the localization of PCM1 immunoreactivity, pericentriolar material was apparently associated with the Balbiani body at the side facing the nucleus.

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Figure 1. A summarized schematic view of asymmetrically located Balbiani body (B) and intercellular bridges during early formation of primordial follicles. Golgi circular structure is surrounded by microfilament ring. N marks the nucleus.

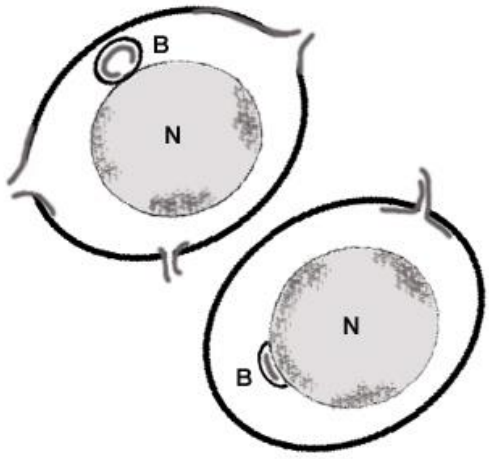

Figure 2. A summarized schematic view of primordial follicle. The oocyte is surrounded by pre-granulosa cells. The pericentriolar material forms double cluster near the oocyte nucleus (N) and the mitochondrial material is colocalized with it but the fluorescence for VDAC1 is more intensive (shown by a dashed line). Both PCM1 and VDAC1 are visible in pre-granulosa cells with perinuclear position and their registration depends on the plane of the section.