Imaging the communicating structures on oocyte through high-resolution spinning-disc microscope

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Method Article

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Abstract

The communications between oocyte and granulosa cells are essential for ovarian folliculogenesis in mammals, and the communicating structures in the ZP had been widely reported. However, there was lack of direct approach to visualize these structures in a 3D model with a high-resolution. Here, we present a protocol to image the structures which derived from oocytes by combining endogenous fluorescent mouse models with a high-resolution spinning disc-confocal system in details

Introduction

As a conserved structure in the zona pellucida, communicating microvilli or transzonal projections (TZPs) have been well documented to exist between oocytes and granulosa cells (GCs) in ovarian follicles of different species including the mammals. However, the origin and the detail structure of these communicating structures are elusive since lacking of cell specific labeling method and high-resolution imaging approach. To visualize the microvilli structures and distinguish oocytic cellular origin during follicle development, we used an mT/mG reporter mouse model[1] in which membrane-localized GFP (mG) is inducible expressed in Cre-positive cells by crossing with oocyte-specific Gdf9-Cre mice[2]. We established a 3D high-resolution imaging system to distinguish the derivation of microvilli by drawing the outlines of the cellular membrane with mG.

Reagents

Mice

Gdf9-Cre (*Gdf9-icre*; JAX 011062)

ROSA $^{mT/mG}$ mice (*mT/mG*; JAX 007576)

Sample preparation and culturing:

MEM α, nucleosides, GlutaMAX™ Supplement (Gibco, 32-571-036)

Fetal Bovine Serum (Gibco, 10-099-141)

ITS Liquid Media Supplement (100×) (Sigma-Aldrich, 13146)

100 U/ml penicillin and 100 μg/ml streptomycin (Thermo Fisher Scientific, 15140122)

PBS (Gibco, 20-012-027)

Mineral oil (Sigma-Aldrich, M8410)

Equipment

Sterile 1 mL syringe (BD, 300841)

Omnican U-40 Insulin syringe (B.Braun, 9161635S)

Mouth pipette for handling and denudation of oocytes

Leica bright field dissection microscope (MZ10)

35 mm culture dish (Nest, 706001)

35 mm glass bottom dish (Cellvis, D35-14-1.5-N)

Andor Dragonfly 502 spinning-disk confocal with living cell workstation (Okolab)

Procedure

1. Generation of *Gdf9-Cre;mTmG* mice:

(1) To generate the *Gdf9-Cre;mTmG* mouse models, adult healthy *Gdf9-Cre* males were crossed with *mT/mG* female mouse in 1:2.

(2) DNA of mouse tail extracted and genotyped to identify the *Gdf9-Cre;mTmG* female before postnatal day (PD) 7.

Gdf9-Cre primers: TCTGATGAAGTCAGGAAGAACC

GAGATGTCCTTCACTCTGATTC

mT/mG primers: CTCTGCTGCCTCCTGGCTTCT

CGAGGCGGATCACAAGCAATA

TCAATGGGCGGGGGTCGTT

2. Preparation of culture medium:

Medium α (MEMα; Gibco) was supplied with 10% FBS (Gibco), 1% insulin-transferrin-selenium (ITS; Sigma-Aldrich) and 0.1% penicillin and streptomycin (Thermo Fisher Scientific), and was pre-warmed to 37℃ before using.

3. Collecting the denuded oocytes:

(1) Ovary was collected from *Gdf9-Cre;mTmG* females at PD 35.

(2) Remove the ovarian bursa carefully by sterile 1 mL syringe in cold PBS buffer.

(3) By tearing the ovaries with Omnican U-40 insulin syringe, denuded oocytes at GV stages were collected in the culture medium. Only the healthy oocytes with an average diameter around 65 μm were collected for the further experiments.

(4) Washing the collected oocytes three times with ~50 μL culture medium microdroplet by mouth pipette gently.

*Tips: Transferring the oocytes gently to guarantee the integrity of ZP.

4. Imaging the cellular membrane structure on living oocytes:

(1) Transfer around 10 oocytes to the culture medium microdroplet (~20 μL) in 35 mm glass bottom dish.

(2) Covered the microdroplet with 1.5 mL mineral oil (Sigma-Aldrich).

(3) Photographed in a living cell workstation (Okolab) at 37°C, 5% CO_2 by Andor Dragonfly 502 spinning-disc confocal with following index. In details, images were acquired by an Andor Dragonfly 502 spinning-disc confocal microscope equipped with a 63×, 1.40 N.A. oil objective (Leica HC PL APO), a scientific complementary metal-oxide semiconductor (sCMOS) camera (Andor Zyla 4.2), and 488-nm (mG) and 568-nm (mT) lines of the Andor Integrated Laser Engine (ILE) system with a spinning-disc confocal scan head (Andor Dragonfly 500). The oocytes were acquired through z-step mode with the index. In detail, images were acquired with laser 488-nm around 10-20%, laser 568-nm around 15-25%, exposure time 100-200 ms and Z-step 0.5-0.7 μm for 25-35 μm by Fusion 2.1 software.

(https://andor.oxinst.com/products/dragonfly#fusion).

(4) After acquisition, videos or single time-point images were processed by ImageJ (http://rsbweb.nih.gov/ij/) for projection of all z-stacks and merged color channels.

(5) To finally detect the Oo-Mv on oocyte, the rotary 3D video was processed by Imaris (https://imaris.oxinst.com/) software.

*Tips: The index of imaging might be changed with the situations of microscope such as the environment temperature and humidity.

Troubleshooting

The index of imaging might be changed with the situations of microscope such as the environment temperature and humidity.

Time Taken

About 2-4 h (except for breeding mice)

References

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