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Dynamics of zonula occludens-2 expression during preimplantation embryonic development in the hamster

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Abstract

The objective was to study the expression of zonula occludens-2, a tight junction protein, during preimplantation hamster embryonic development, to predict its possible localization, source, and roles in trophectoderm differentiation and blastocyst formation in this species. Comparison of zonula occludens-2 expression pattern between the hamster and mouse preimplantation embryos from the zygote up to the blastocyst stage was also an objective of this study. Zonula occludens-2 localization was noted in nuclei of blastomeres in all stages of hamster and mouse embryonic development. Compared to mice, where zonula occludens-2 was first localized in the interblastomere membrane at the morula stage, hamster embryos had membranous zonula occludens-2 localization from the 2-cell stage onwards. Based on combined results of immunolocalization study in parthenogenic embryos and ovarian and epididymal sections, and quantitative PCR done in oocytes and all developmental stages of preimplantation embryos, perhaps there was a carry-over of zonula occludens-2 proteins or mRNA from the dam to the embryo. Based on these findings, we inferred that maternally derived zonula occludens-2 was involved in nuclear functions, as well as differentiation of blastomeres and blastocoel formation during preimplantation embryonic development in the hamster.

Keywords: Zonula occludens-2; Preimplantation embryo; Oocyte; Hamster; Mouse

1. Introduction

Blastocyst formation occurs as a result of zygotic division and blastomere differentiation. The nascent blastocyst contains two committed cell subpopulations, the polarized trophectoderm (TE), and the pluriblast or inner cell mass (ICM). The establishment of these two cell lineages is critical, since the TE is required for initiation of implantation and placenta formation, whereas the ICM is required for formation of the fetus and extra-embryonic endodermal lineages. Blastomere differentiation to form the TE is a progressive process, and initiates from the 8-cell stage in the mouse conceptus. Adherens and tight junction (AJ and TJ) protein accumulation at the blastomere to blastomere contact sites from the 8-cell stage onwards is a prerequisite for normal TE formation [1–3].

Recently, we reported that the hamster conceptus became a blastocyst at the 16-cell stage, one cell cycle ahead of mice [4]. This early blastocyst formation phenomenon in hamsters motivated us to investigate TJ

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protein localization during zygotic development in this species. The tight junction (TJ) is the apical-most intercellular junction of the epithelial cell layer; it is responsible for maintenance of intramembranous apical-basal polarity (fence function) and creation of a physiological barrier (gate function) that limits the movements of solute and solvents in between cells. The TJ is formed when transmembrane proteins such as occludin and claudins bind to actin filaments via zonula occludens (ZO) proteins, ZO-1, ZO-2, and ZO-3 [5,6]. Since murine embryos null for ZO-2 died at gastrulation, perhaps it was not required for blastocyst formation in mice [7]. However, an siRNA-mediated ZO-2 knockdown approach in mouse embryos delayed blastocyst formation [8]; perhaps there was a ZO protein redundancy as a compensatory mechanism for blastocyst formation. However, zonula occludens-2 not only served as an organizer of TJ, but was also involved in transcriptional regulation, and cell proliferation and differentiation [6]. Similarly, blastomeres of the embryo not only undergo proliferation like their somatic brethren, but also differentiate to form TE. Gene transcription and translation for blastomere division and differentiation occur for the first time during zygotic development. Therefore, expression patterns of ZO-2 in cleaving hamster concepti were investigated in this study to determine its possible function during preimplantation embryo development.

2. Materials and methods

2.1. Eggs and embryo collection

Golden hamsters and CD-1 mice were purchased from Charles River Laboratories (Wilmington, MA, USA) and kept in a light-dark cycle (12 h light:12 h dark) in the Laboratory Animal Facility of the Vanderbilt University Medical Center (Nashville, TN, USA), with *ad libitum* access to water and food, according to the Institutional Guidelines on the Care and Use of Laboratory animals. The Vanderbilt University Institutional Animal Care and Use Committee approved all procedures.

Female hamsters were paired with males on the evening of proestrus for mating, except for females used for oocyte collection. The presence of sperm in vaginal secretions of hamsters the next morning indicated Day 1 of pregnancy. Female mice were caged with fertile males overnight for mating and the presence of a copulation plug the following morning indicated Day 1 of pregnancy. Oocytes and various stages of preimplantation embryos from hamsters and mice were collected by flushing oviducts or uteri with hamster embryo culture medium-9 (HECM-9), or potassium simplex optimized medium with amino acids (KSOMaa), respectively [9].

2.2. Embryo whole-mount immunofluorescence

Oocytes and embryos were fixed in cold paraformaldehyde (2%), permeabilized, preincubated with serum of the species from which the second antibody was derived, incubated with primary antiserum (a rabbit polyclonal anti-ZO-2 (cat. no. 71-1400; Invitrogen Corporation, Carlsbad, CA, USA) overnight at 4 °C, washed, and incubated again with FITC-conjugated goat anti-rabbit IgG (cat. no. 81-6111; Invitrogen Corporation) for 2 h at room temperature. Embryos were then stained with 4, 6′-diaminido-2-phenylindole, dihydrochloride (DAPI; cat. no. D9542; Sigma-Aldrich Corporation, St. Louis, MO, USA) to visualize nuclei. Non-specific staining was determined by processing embryos as described above, in the absence of primary antiserum. Embryos were mounted on a glass slide with Fluoromount G (cat. no. 0100-01; SouthernBiotech, Birmingham, AL, USA) and localization of ZO-2 was visualized under a Nikon Eclipse TE2000E inverted microscope equipped with X-Cite 120 for fluorescence and Opti-grid structured light confocal imaging system (Boyce Scientific, Inc., Gray Summit, MO, USA) [10].

2.3. Ethanol-induced parthenogenetic activation of oocyte

Hamsters were given PMSG (25 IU, ip; Sigma-Aldrich Corporation) and hCG (5 IU, ip; Sigma-Aldrich Corporation) to induce superovulation. Oocytes were collected approximately 17 h after hCG treatment, exposed to 7% ethanol for 5 min for parthenogenetic activation, and cultured overnight in HECM-9 [11,12]. Ethanol-activated oocytes containing two maternal pronuclei and cleaved to 2-cell stage embryos were processed for ZO-2 immunostaining, to determine whether nuclear ZO-2 in early embryos was maternally inherited.

2.4. Quantitative real-time polymerase chain reaction (qPCR)

Three independent batches of oocytes and various stages of developing preimplantation embryos (13 oocytes or embryos/group) were collected for isolation of total RNA, using the method previously described [10]. Total RNA from each batch was reverse transcribed with oligo-dT primer, and the RT-derived cDNA was then subjected to qPCR (LightCycler 2.0,