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Lipid-rich blastomeres in the two-cell stage of porcine parthenotes show bias toward contributing to the embryonic part

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ABSTRACT

This study was designed to determine the fate of the blastomeres in two-cell porcine parthenotes that display uneven size (larger vs. smaller) or cytoplasmic brightness (darker vs. brighter) during development to the blastocyst stage. For the non-invasive tracing of cell lineage, lipophilic fluorescence dye DiI (red) and DiD (blue) was randomly microinjected into each of two different blastomeres in each embryo. In blastocysts derived from the two-cell parthenotes with unevenly-sized blastomeres, no biased contribution was found in the progeny of either blastomere. However, in the blastocysts derived from the two-cell parthenote having different cytoplasmic brightnesses, the progeny of darker (more lipid-rich cytoplasm) blastomeres were more than two-fold more likely to form the embryonic part (43.6%; 17/39) than they were to form the abembryonic part (17.9%; 7/39), while the contribution of brighter blastomeres (less lipid) was just the opposite. The expressions of four marker genes involved in lineage allocation (*Cdx2*, *Tead4*, *Oct4* and *Carm1*) were also analyzed in darker and brighter blastomeres of two-cell parthenotes using quantitative RT-PCR. The expression of *Carm1* that encodes arginine methyltransferase 1 and that promotes inner cell mass (ICM) differentiation was significantly higher ($P < 0.05$) in darker blastomeres. The ICM marker *Oct4* also tended to be more highly expressed in the darker blastomeres, while *Cdx2* and the TE marker *Tead4* showed comparably higher expressions in the brighter blastomeres. However, in all cases, the marginal differences in the expression levels of *Oct4*, *Cdx2* and *Tead4* were not statistically significant ($P > 0.05$). Our findings indicate that expression of genes related to early differentiation, especially *Carm1*, are partially associated with lipid droplet distribution in the two-cell porcine parthenote and may lead to biased embryonal axis formation.

1. Introduction

In mammalian embryogenesis, early single blastomeres are able to develop into either inner cell mass (ICM) or trophectoderm (TE) of the blastocyst. However, their totipotent ability is gradually decreased with further development. During early mammalian embryonic development, Octamer-binding transcription factor 4 (Oct4), also known as Pou5f1, is expressed in the ICM cells in the blastocyst and promotes ICM differentiation (Loh et al., 2006; Niwa et al., 2005; Oestrup et al., 2009). TEA domain family transcription factor (Tead4) is a key regulator of blastocoel formation and of specification of TE in preimplantation mouse embryos. *Tead4*-null mouse embryos fail to express TE-specific genes, such as *Caudal-related homeobox 2* (*Cdx2*), *aPKC* and *Eomesodermin* (*Eomes*), while the ICM markers, *Oct4* and *Nanog*, are expressed in all blastomeres (Nishioka et al., 2008; Yagi et al., 2007). Cdx2 is also required for specification of TE in porcine and bovine embryos (Kuijk et al., 2008; Niwa et al., 2005). Cdx2

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regulates E74-like factor 5 (Elf5) and Eomes, while it inhibits the expressions of the ICM-specific genes *Oct4* and *Nanog*. In *Cdx2*-null blastocysts, Oct4 and Nanog are detected in TE cells. In the morula stage, Cdx2 is more highly expressed in outer blastomeres than it is in inner ones, and outer blastomeres subsequently contribute to TE, while inner ones contribute to ICM (Jedrusik et al., 2008; Strumpf et al., 2005).

In mice, single blastomeres at the two-cell stage can develop into adult mice (Papaioannou et al., 1989; Tarkowski, 1959). Furthermore, the 4- to 32-cell stage murine blastomeres are also able to develop into a fetus or even into a live baby when tetraploid embryos are used as the carrier. In contrast, the first cell fate decision has been reported to exist in the two- or four-cell stage blastomere in several cases. In mouse embryos, the meridionally dividing blastomere tends to contribute the embryonic part of the fetus and the other blastomere is abembryonic (Piotrowska-Nitsche and Zernicka-Goetz, 2005) and expresses higher levels of coactivator-associated arginine methyltransferase 1 (*Carm1*), while the other blastomere more highly expresses *Cdx2* (Jedrusik et al., 2008; Torres-Padilla et al., 2007). Carm1, which methylates arginine 17 and 26 on histone H3 (H3R17 and R26), leads to blastomere translocation to the inside of the mouse embryo. Increased expression of *Carm1* upregulates *Nanog* and *Sox2* and down-regulates *Cdx2*, therefore promoting ICM differentiation (Henckel et al., 2007; Parfitt and Zernicka-Goetz, 2010; Torres-Padilla et al., 2007).

In *Sminthopsis macroura* embryos, asymmetrical distribution of lipid droplets is observed and influences lineage allocation (Au et al., 2010). In *Drosophila* embryonic development, lipid droplets store histones and proteins that are related to zygotic genome activation. Lipid droplets may play a role in the maintenance of maternal histones and proteins in an inactivate state until they are needed because overexpression of histones induces abnormal embryogenesis (Berloco et al., 2001; Cermelli et al., 2006). Lipid storage droplet protein 2 (LSD2) is a member of the family of perilipin/ADRP/TIP47 (PAT) proteins and regulates lipid droplet transport and subsequently controls the apical-basal axis formation (Au et al., 2010; Cermelli et al., 2006; Welte et al., 2005). Lipid droplets of the mammalian oocyte are observed as dark spots, while porcine oocytes have a darker ooplasm and greater abundance of total lipids than do bovine, murine, human and sheep oocytes (Genicot et al., 2005; Loewenstein and Cohen, 1964; McEvoy et al., 2000; Sturmey and Leese, 2003). Lipid droplets in mammalian embryos are mainly considered to function in energy metabolism during oocyte maturation, fertilization, and early embryogenesis (Kikuchi et al., 2002). However, the role of lipid droplets in early embryonal axis formation in mammals is poorly understood.

We previously demonstrated that the first dividing blastomere of the two-cell stage porcine parthenote tends to contribute to TE, while the other blastomere contributes to ICM (Park et al., 2009). Porcine polyspermic fertilization is a major problem of in vitro insemination (Kouba et al., 2000). To avoid the influence of the fertilization cone via polyspermic fertilization, parthenogenetic embryos were used in this study, and the embryonal axis formation was investigated when the two-cell porcine parthenotes showed uneven size or cytoplasmic brightness (different amounts of lipid droplets) between blastomeres. For non-invasive lineage tracing of the blastomere, DiI (red) or DiD (blue) lipophilic fluorescence dye was randomly injected into each blastomere of the two-cell stage parthenote. The contribution of the progeny of each blastomere to either ICM or TE was then assayed at the blastocyst stage using confocal microscopy. The expression levels of genes related to the embryonal lineage decision (*Cdx2*, *Tead4*, *Oct4* and *Carm1*) were also analyzed using real-time PCR. All inorganic and organic compounds were obtained from Sigma–Aldrich, Korea (Yong-in, Korea), unless otherwise stated.

2. Materials and methods

2.1. Oocyte recovery and in vitro maturation (IVM)

Slaughterhouse ovaries were collected from 5- to 6-month-old prepubertal gilts (100 ± 10 kg body-weight), placed in saline at 30–35 °C, and transported within 2 h to the laboratory. After washing with saline three times, cumulus-oocyte complexes (COCs) were recovered via aspiration of 2- to 5 mm follicles using an 18-gauge hypodermic needle attached to a 5 ml disposable syringe. After washing three times in IVM medium, COCs that were enclosed in more than three layers of compact cumulus cells and an evenly granulated ooplasm were selected for IVM. Selected COCs were cultured in four-well culture dishes (Nunc, Denmark) containing 500 μl of IVM medium under warmed and gas-equilibrated mineral oil for 46–48 h at 38.5 °C and 5% CO_2. The IVM medium for oocytes was composed of tissue culture medium 199 with Earle's salts and L-glutamine (TCM199, Gibco Life Technologies Inc., USA) supplemented with 26.2 mM $NaHCO_3$, 3.05 mM glucose, 0.91 mM sodium pyruvate, 0.57 mM L-cysteine, 10 ng/ml epidermal growth factor, 10 IU/ml equine chorionic gonadotropin and human chorionic gonadotropin, and 0.1% (v/v) polyvinylalcohol (PVA), as previously described (Koo et al., 2005).

2.2. Parthenogenesis and in vitro culture

Electrical activation was performed at room temperature using a CF-150/B electro-cell fusion system (BLS, Hungary) in a chamber that contained two stainless steel electrodes that were 1.0 mm apart and that was filled with activation buffer. Oocytes were activated with a 1.6 kV/cm DC pulse for 40 μs in 0.26 M mannitol supplemented with 0.1 mM $MgSO_4$, 0.05 M $CaCl_2$, and 0.01% PVA. The activated oocytes were treated for 5–6 h in NCSU-23 supplemented with 5 μg/ml cytochalasin B. The oocytes were then washed nine times with NCSU-23 and cultured in 20 μl drops (10–15 oocytes per drop) of NCSU-23 at 38.5 °C and 5% CO_2.

2.3. Fluorescent labeling of each blastomere

After 22–24 h of activation, the two-cell porcine parthenotes were sorted into either uneven size (larger