INHIBITION OF MEK1/2 AND GSK3 (2I SYSTEM) AFFECTS EPIGENETIC MODIFICATION AND EARLY DIFFERENTIATION OF PORCINE PARTHENOTES

Jeongwoo Kwon[1], Yu-Jin Jo[1], Taoan Kim[2], Suk Namgoong[1], Nam-Hyung Kim[1]*

[1]Department of Animal Science, Chungbuk National University, Cheongju, Chungbuk, 28644 Korea

[2]Department of Physiology, Catholic University of Daegu School of Medicine, Daegu 705718 Korea.

Introduction

Preimplantation development of mammalian embryos features several embryonic differentiations that yield the fetus and placenta. In the morula stage of embryos, first cell lineage determinations occur, and cells in an embryo differentiate as the inner cell mass (ICM) or trophectoderm (TE) cells. Embryonic stem cells (ESCs) can be isolated from the ICM in blastocysts prior to the differentiation to the three embryonic germ cell layers (ectoderm, endoderm, and mesoderm). Inhibition of both MEK1/2 and GSK3 (2i system) facilitates the maintenance of naïve stemness for embryonic stem cells in various mammalian species. However, the effect of the inhibition of the 2i system on porcine early embryogenesis and epigenetic modifications is unknown. We investigated the effect of the 2i system on early embryo development, pluripotent gene expressions, and epigenetic modification.

Materials and Methods

Aspiration of follicle fluid from porcine ovaries were done using an 18-gauge needle with a 10 mL syringe. COCs were cultured in a 4-well cell culture dish with in vitro maturation medium. Denuded oocytes were washed three times in PBS-BSA and activated using an Electro Cell Manipulator 2001 (BTX, Inc., San Diego, CA, USA) on 280 mM mannitol medium supplemented with 0.01 mM CaCl2 and 0.05 mM MgCl2. Activated oocytes were placed on PZM-5 medium containing 7.5 μg/mL cytochalasin B for 3 h. Embryos were washed three times and cultured in PZM-5 medium containing dimethylsulfoxide (DMSO, 0.2%; Control group), MEK1/2 inhibitor (PD0325901, 4 μM), GSK3 inhibitor (CHIR99021, 0.3 μM), or 2i inhibitor (PD0325901 4 μM + CHIR99021 0.3 μM), for 144 h at 38.5°C in an atmosphere containing 5% CO2.

Results and Discussion

Inhibition of MEK1/2 (by PD0325901) and/or GSK3 (by CHIR99021) did not change the developmental potential of porcine parthenogenetic embryo blastocysts, but improved blastocyst quality, judged by the blastocyst cell number, size, and decreased number of apoptotic cells. Expression levels of OCT4 and SOX2, the primary transcription factors that maintain embryonic pluripotency, were significantly increased by 2i treatments. Epigenetic changes, including decreased Histone 3 Lysine 9 trimethylation (H3k9me3), increased Histone 3 Lysine 9 acetylation (H3k9ac), and increased demethylation at the satellite I regions were observed upon 2i treatment. The collective results indicate that 2i system in porcine embryos improved blastocyst quality by regulating of epigenetic modification and pluripotent related gene expression.

*Supported by Next Generation Biogreen 21 Program (PJ01322101), Rural Development Administrations, Republic of Korea