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Short Communication

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Tight Junction Assembly Ensures Maintenance of Pregnancy during Embryogenesis in a Mouse Model

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ABSTRACT Recent studies showed that tight junctions (TJs) integrity and assembly are required for blastocyst development in mouse and pig models. However, the biological functions of TJs associated with embryo implantation and maintenance of pregnancy were not investigated yet. To examine whether disrupted TJs affect further embryo development, we employed RNAi approach and inhibitor treatment. The embryos were injected with Cxadr (Coxsackievirus and adenovirus receptor) siRNA for knock down (KD) and treated with Adam10 (A Disintegrin and Metalloproteinase specific inhibitor 10; GI254023X; SI). We compared blastocyst development and paracellular sealing assay using FITC dextran uptake between control and KD or SI embryos. Finally, we transferred control and Cxadr KD or Adam 10 SI treated blastocyst to uteri of recipients. Cxadr KD and Adam 10 SI showed lower blastocyst development and more permeable to FITC-dextran. Moreover, we observed that half of KD and inhibited embryos failed to maintain pregnancies after the second trimester. Our findings suggested that TJs integrity is required for the maintenance of pregnancy and can be used as a selective marker for the successful application of assisted reproduction technologies.

Keywords: Adam10, blastocyst, Cxadr, embryo transfer, tight junction

INTRODUCTION

A fertilised zygote undergoes rapid mitotic division to form a blastocyst that consists of an inner cell mass (ICM) and an outer trophectoderm (TE) layer, and a fluid-filled cavity (a blastocoel). Tight junctions (TJs) biogenesis are responsible for formation of the blastocyst cavity during morula to blastocyst transition (Watson and Barcroft, 2001; Cockburn and Rossant, 2010). The significance of tight intersection (TJ) and cavitation during preimplantation embryo development have been featured since McLaren and Smith's pioneer work (McLaren and Smith 1977). Recent studies revealed that Tfap2c is a core key transcription factor for establishment of TJ complex in mouse models, and identified other constituents involved in TJ assembly during preimplantation (Choi et al., 2012; Kwon et al., 2016a; Kwon et al., 2016b). In addition, we reported that complex of coxsackievirus and adenovirus receptor (Cxadr) and A disintegrin and metalloproteinase 10 (Adam10) play pivotal roles in TJ integrity/stability and early trophoblast development in mouse. However, there is no clear evidence that incomplete or defects of TJ as-

sembly/complex affect post-implantation development.

To explore potential use of TJ complex integrity as an embryo mortality related bio-marker, we expand our previous finding where CXADR is involved in AJ/TJ formation and ADAM10 interacts with CXADR and TJP1 for is sequential TJ assembly during preimplantation development (Jeong et al., 2019). We first examined preimplantation developmental competency by deletion of components of TJ complex injecting Cxadr (Coxsackie virus and adenovirus receptor) siRNA or using disruption/inhibition of TJ complex using a chemical inhibitor of Adam 10 (GI254023X). Next, we investigated maintenance of pregnancy following ET (embryo transfer) to determine whether disrupted/incomplete TJ affects implantation and further development.

MATERIALS AND METHODS

All the animal studies were approved by Institution Animal Care and Use Committee guidelines from the Chungnam National University Animal Welfare and Ethical Review Body (License No. CNU-00702).

Embryo culture, micromanipulation

Mouse embryos were obtained, cultured, and manipulated as previously described (Jeong et al., 2019). Female mice (6-8 week-old B6D2/F1; KOATECH, Pyeongtaek, Republic of Korea) were superovulated by PMSG and hCG injection, and then they were mated with males (B6D2/F1). Zygote embryos were collected in M2 medium (Sigma-Aldrich, St. Louis, MO, USA) at 16 hours after mating. For knock down (KD) experiments, 100 μM mouse Cxadr siRNA (siGenome; Dharmacon, Lafayette, CO, USA) and 100 μM control non-target siRNA (Dharmacon) was injected into the cytoplasm of zygotes using a PLI-100A Pico-Injector (Harvard Apparatus, Holliston, MA, USA). Following injection, embryos were cultured in modified KSOM medium (EMD Millipore, Billerica, MA, USA) until use. Morula embryos were incubated in the culture medium supplemented with 100 μM GI254023X (Sigma-Aldrich) for 24 h in order to determine effect of selective ADAM 10 inhibition (SI) on TJ complex/integrity and blastocyst development.

TJ permeability assay

Control and KD or SI treated blastocysts were incubated with 4 kDa FITC-dextran (1 mg/mL; Sigma-Aldrich) for 10 minutes in the culture medium. The treated blastocysts were immediately washed three times with M2 medium and placed in a clean M2 medium drop for assessment of FITC-uptake under an epi-fluorescent microscope (Nikon Eclipse Ti-U, Nikon).

Embryo transfer (ET)

Control and KD or SI treated blastocysts were transferred to the uterus of pseudo-pregnant recipients (2.5 days post coitum; d.p.c). To compare implantation and maintenance of pregnancies, for each recipient, control blastocysts (n = 10) were transferred to one uterine horn, and Cxadr KD or Adam10 inhibitor treated blastocysts

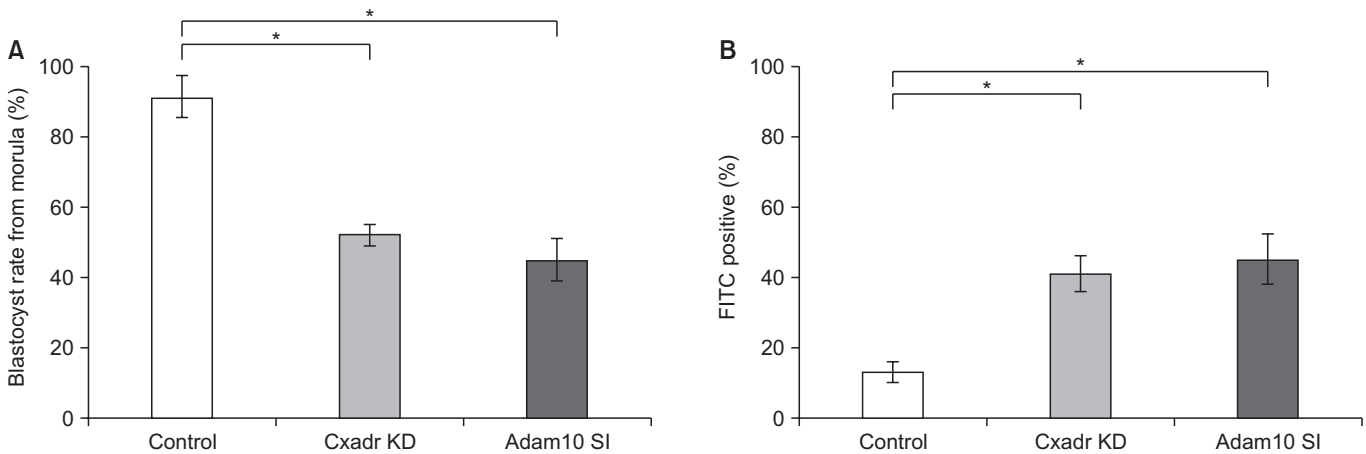

Fig. 1. Effects of knock-down (KD) of Cxadr or Adam10 selective inhibitor (SI) treatment on mouse embryo development. (A) Cxadr KD and Adam10 SI embryos showed lower blastocyst development. (B) Significant differences in FITC-dextran uptake between control and Cxadr KD/Adam 10 SI blastocyst. Student's t test *$p < 0.05$. Three biological replicates.

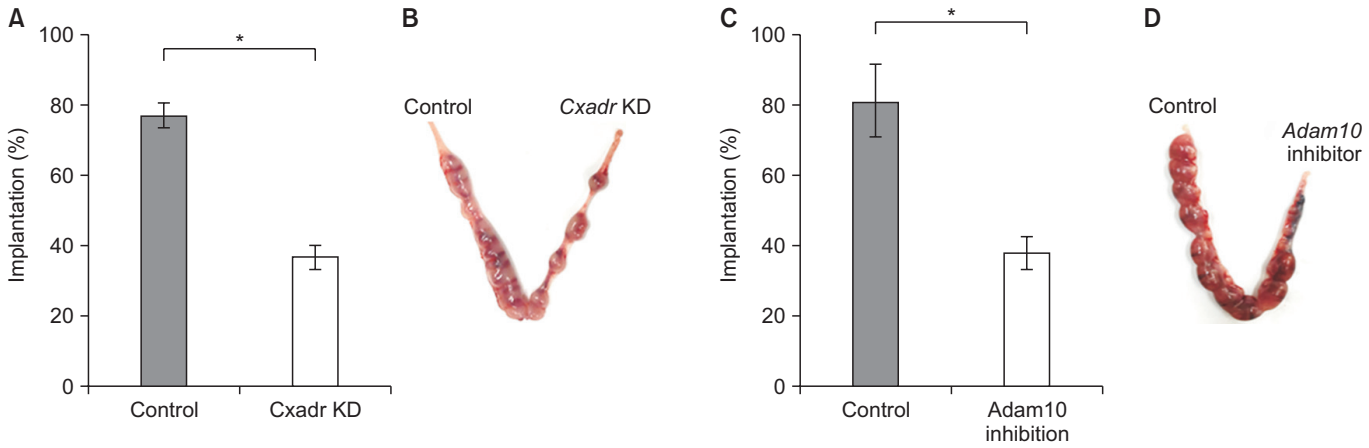

Fig. 2. Effects of Cxadr KD and Adam inhibitor treatment on postimplantation development. (A) Implantation rates. Control (n = 30) and Cxadr (n = 30) blastocyst were transferred equally into two uterine of pseudopregnancy, and implantation rates were measured at 9 days after embryo transfer (ET). (B) Representative images of uterus in sacrificed mouse that received control and Cxadr KD blastocysts. (C) Control (n = 30) and Adam10 inhibitor treated blastocysts (n = 30) were transferred equally into two uterine of pseudopregnancy, and implantation rates were measured at 9 days after ET. (D) Representative images of uterus in sacrificed mouse that received control and Adam10 selective inhibitor treated blastocysts. Student's t test *$p < 0.05$.

(n = 10) placed into another horn. Total three biological replicates were performed for the KD and the inhibitor treatment group. Body weight of recipients was measured after ET. The females were sacrificed when the weight was reduced in two consecutive days.

Statistical analysis

Data were analysed by Student *t*-test or analysis of variance (ANOVA) using GraphPad Prism (Version 5.03, GraphPad Software, San Diego, CA, USA). The data are presented as mean ± s.e.m. (standard error mean). *p* values <0.05 were considered statistically significant differences unless otherwise stated.

RESULTS AND DISCUSSION

In agreement with a previous study (Jeong et al., 2019), Cxadr KD resulted in retardation of blastocyst (52 ± 3%, Fig. 1A), and morula embryos treated with selective inhibition (SI) of Adam 10 also arrested and developed blastocyst (45 ± 6%, Fig. 1A) had small cavity without expansion. In addition, paracellular sealing assay using FITC dextran uptake showed that the Cxadr KD (41 ± 5%) and Adam 10 (45 ± 7%) SI blastocysts more permeable than control blastocyst (13 ± 3%) (Fig. 1B).

Next, control, *Cxadr* KD, and Adam 10 SI treated blastocysts were transferred into surrogates in order to examine embryonic loss during post-implantation development. Control blastocysts were placed into one side of the oviducts, and *Cxadr* KD or Adam10 SI blastocysts were transplanted into the other side. The pseudopregnancy recipient mice were sacrificed following weight-loss for two consecutive days. The weight-losses were not observed until 7 days after ET (embryo transfer). Interestingly, implantation sites were maintained in uteri receiving control blastocysts (~80%), but the sites were dropped by nearly half (37%) in uteri receiving the *Cxadr* KD and Adam 10 SI blastocyst (Fig. 2).

The purpose of this study is to investigate the effect of defects of TJ complex/integrity on postimplantation embryo development and to expand our previous findings (Kwon et al., 2016a; Kwon et al., 2016b; Jeong et al., 2019) where CXADR interacts with ADAM 10 and they are required for AJ/TJ assembly during preimplantation development in pig and mouse. In line with previous studies (Kwon et al., 2016a; Kwon et al., 2016b; Jeong et al., 2019), we found that depletion of Cxadr and inhibition of Adam10 resulted in developmentally arrest during morula and blastocyst transition, and the embryos developed to blastocyst had defective TJs. To examine the effects of impaired TJ complex on postimplantation development including implantation and maintenance of pregnancy, embryo transfer (ET) was performed. We measured maternal body weight every day after ET to assess implantation and pregnancy status because maternal body weight increases progressively after mating (Kulandavelu et al.,

2006). Thus, the gradual weight gain after ET may reflect increased embryo and placental weight, but weight-loss for two consecutive days is attributed to loss of embryos because maternal body weight rapidly increased from around 2nd trimester onwards (Kulandavelu et al., 2006). Therefore, lower number of implantation sites in the Cxadr KD and Adam10 SI treated group might reflect maintenance of pregnancy rather than conception/implantation.

CONCLUSION

Taken together, our findings support the importance of tight junction and critical functional roles for trophoblast development and further development (Sivasubramaniyam et al., 2013; DaSilva-Arnold et al., 2016; Mobley et al., 2017).

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

Choi I, TS Carey, CA Wilson, and JG Knott. 2012. Transcription factor AP-2gamma is a core regulator of tight junction biogenesis and cavity formation during mouse early embryogenesis. Development. 139:4623-4632.

Cockburn K, and J Rossant. 2010. Making the blastocyst: lessons from the mouse. The Journal of Clinical Investigation. 120:995-1003.

DaSilva-Arnold S, S Zamudio, A Al-Khan, and N Illsley. 2016. The tight junction protein occludin is regulated by RhoA and activin A in JEG3 trophoblast cells. Placenta. 45:116.

Jeong Y, S-A Ock, JG Yoo, D-Y Yu, and I Choi. 2019. The Cxadr–Adam10 complex plays pivotal roles in tight junction integrity and early trophoblast development in mice. Molecular Reproduction and Development. https://doi.org/10.1002/mrd.23250.

Kulandavelu S, D Qu, N Sunn, J Mu, MY Rennie, KJ Whiteley, JR Walls, NA Bock, JCH Sun, A Covelli, JG Sled, and SL Adamson. 2006. Embryonic and Neonatal Phenotyping of Genetically Engineered Mice. ILAR Journal. 47:103-117.

Kwon J, N-H Kim, and I Choi. 2016b. CXADR is required for AJ and TJ assembly during porcine blastocyst formation. Reproduction. 151:297-304.

Kwon J, S Jeong, I Choi, and NH Kim. 2016a. ADAM10 Is Involved in Cell Junction Assembly in Early Porcine Embryo Development. PLoS One. 11:e0152921.

McLaren A, and R Smith. 1977. Functional test of tight junctions in the mouse blastocyst. Nature. 267:351.

Mobley RJ, D Raghu, LD Duke, K Abell-Hart, JS Zawistowski, K Lutz, SM Gomez, S Roy, R Homayouni, GL Johnson, and AN Abell. 2017. MAP3K4 Controls the Chromatin Modifier HDAC6 during Trophoblast Stem Cell Epithelial-to-Mesenchymal Transition. Cell Reports. 18:2387-2400.

Sivasubramaniyam T, J Garcia, A Tagliaferro, M Melland-Smith, S Chauvin, M Post, T Todros, and I Caniggia. 2013. Where Polarity Meets Fusion: Role of Par6 in Trophoblast Differentiation during Placental Development and Preeclampsia. Endocrinology. 154:1296-1309.

Watson AJ, and LC Barcroft. 2001. Regulation of blastocyst formation. Front Biosci. 6:D708-730.