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Effects of CD26 in Parthenogenetically Activated Porcine Embryos

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ABSTRACT

CD26, also known as Dipeptidyl peptidase IV (DPP-4), is a cell surface glycoprotein that belongs to the serine protease family and has wide spread organ distribution throughout the body. CD26 was previously characterized in immune cells but also has important metabolic functions which are not yet fully understood. Thus, we investigated the effect of CD26 in porcine parthenogenetic embryos. We attempted CD26 downregulation of porcine embryos by siRNA, and evaluated CD26 suppression of developmental competencies. Although the porcine embryos injected with CD26 siRNA were able to develop to the early stage, these embryos were decreased to form blastocysts. Our results indicated that CD26 is one of factors for the regulation of development of porcine embryos.

(Key words: CD26, *Parthenogenetic embryo*, *Porcine*)

INTRODUCTION

Embryonic development is annotated as a complex event including formation of the embryo, fate determination and differentiation of certain cell types and organogenesis, which are initiated and controlled by numerous regulatory networks.

CD26/DPP IV is a 110-kDa extracellular glycoprotein that is widely expressed in kidney, liver, placenta, renal tubules, epithelial cells (Heike *et al*., 1988; Nemoto *et al*., 1999; Mizutani et al., 1985) and human blastocyst (Shimomura *et al*., 2006). Also, It is highly expressed on the endometrial epithelium during the implantation period in women (Imai *et al*., 1992) and in preeclamptic placentas with intrauterine growth restriction (Nishikawa *et al*., 2005). CD26 have a various function such as regulation of inflammatory, immunological response, signal transduction, and apoptosis (Hanski *et al*., 1985; Loster *et al*., 1995; Gaetaniello *et al*., 1998).

CD26 expression was mostly found in the proximal part of the cell but there have been no reports on the effect of CD26 in porcine embryonic development. The objectives of this study were to assess the knockdown efficiency of CD26 using siRNA during porcine preimplantation embryonic development.

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MATERIALS and METHODS

1. Animal ethics

All animal experiments were approved and performed under the guidelines of the RDA Care and Experimentation Committee.

2. In Vitro Maturation

All chemicals were purchased from Sigma-Aldrich (St. LOUIS, MO, USA) unless indicated otherwise. Briefly, prepubertal pig ovaries were obtained from gilts at a local slaughterhouse and transported to the laboratory in saline at around 30 to 35 ℃ within 1h Cumulus-oocyte complexes (COCs) were aspirated from follicles (3~6 mm diameter) by 18 gauge needle attached to a 10ml disposable syringe. The follicular fluid with COCs were collected into conical tubes and washed 3 times in tissue culture medium (TCM-199) containing 0.1 % (w/v) polyvinyl alcohol (PVA). After sedimentation, the COCs with several layers of compact cumulus cells were selected for in vitro maturation (IVM). After selection, about 50~70 COCs were transferred into 500 ul of TCM-199 medium (Gibco BRL., USA) supplemented 10 % porcine follicular fluid (pFF), 3.05 mM D-glucose, 0.57 mM cysteine, 0.91 mM sodium pyruvate, 0.5 ug/ml FSH, 0.5 ug/ml LH, 10 ng/ml EGF, 75 ug/ml penicillin G and 50 ug/ml

streptomycin in a four-well dish. The COCs were matured for 22 h with hormone and 22 h without hormone at 39 ℃ under 5 % CO_2 in air.

3. Cytoplasmic Injection of CD26 siRNA

After IVM, cumulus cells were removed from oocytes by gentle pipetting after incubating the COCs in PBS supplemented with 0.1 % hyaluronidase and 0.1 % PVA for 5 min. The exogeneous vector were injected into the oocytes with visible first polar body with TCM-199 medium supplemented with 0.59 mM sodium bicarbonate, 3.14 mM Hepes, 30.2 mM sodium chloride, 50 ug/ml penicillin G, 60 ug/ml streptomycin, 5 ug/ml cytochalasin B. Microinjection was carried out using an inverted microscope (Olympus, Tokyo, Japan). The CD26 was injected with 3 groups of Uninjected, Control siRNA and CD26 siRNA treatments. CD26-siRNA (sc-42762) and control-siRNA (sc-37007) were obtained from Santa Cruz Biotechnology (CA, USA).

4. Parthenogenetic Activation and In Vitro Culture

Microinjected oocytes were activated in medium containing 0.3 M mannitol, 1.0 mM $CaCl_2$ and 0.5 mM Hepes. In between 0.2 mm diameter of electrodes, 2 direct current pulses (1 sec interval) of 1.25 kV/cm were applied for 30 usec using an Electro Cell Fusion (NEPA gene, Chiba, Japan). Activated embryos were washed and cultured in porcine zygote medium-3 with four-well dish at 39 ℃ under 5 % CO_2 in air. During 7 days of culture period, the embryos were examined the cleavage, blastocyst formation rates and TUNEL staining.

5. TUNEL assay

The blastocysts on Day 7 were washed twice in PBS/PVP (PBS supplemented with 0.1 % polyvinylpyrolidone) and fixed in 4 % (v/v) paraformaldehyde solution for 24 hr at 4 ℃ . Membranes were permeabilized in 0.5 % Triton X-100 for 30 min at room temperature. A TUNEL assay was used to assess the presence of apoptotic cells (in situ cell death detection kit, TMR red; Roche, Mannheim), for 1 hr at 38.5 ℃ in the dark. The broken DNA ends of the embryonic cells were labeled with TDT and fluorescein-dUTP. After the reaction stopped, the embryos were washed and transferred into 10 ug/ml Hoechest 33342 for 30 min at room temperature. The embryos were washed three times and mounted on slides with Prolong antifade Ket (Cat. P-748, Molecular Probes, Eugene, OR). The slides were stored at －20 ℃. The numbers of apoptotic nuclei and total numbers of nuclei were determined from optical images of whole-mount embryos under an epifluorescent microscope (Nikon, Tokyo, Japan).

6. Statistical analysis

Data were subjected to a Generalized Linear Model procedure (PROC-GLM) of the Statistical Analysis System (SAS Institute, Cary, NC). Differences among treatment means were determined by using the Duncan's multiple rage tests. All data were expressed as Least Square (LS) mean ± SEM (Standard Error of the sample Mean). A probability of $P < 0.05$ was considered statistically significant.

RESULTS and DISCUSSION

MII oocytes were injected with CD26 siRNA or control siRNA and cultured in development medium. The rate of blastocyst formation and blastocyst cell numbers are presented in Table 1, Fig. 1 and Fig. 2. The number of cleaved oocytes was counted 48h after electro-activated and blastocysts were determined 7 days. As shown in Table 1, the average rate of embryos cleavage was 84.4 %, 84.1 % and 85.9 %. Blastocyst rate was 42.2 %, 37.2 % and 10.6 %. Cleavage rate was similar to 3 groups, but blastocyst rate was significantly decreased in CD26 siRNA group (Fig. 1). There were no significant differences among groups in the number of total cell but decreased in CD26 siRNA group (44.5, 48.6 and 35.3). Number of apoptotic cells was increased in siRNA treatment groups (2.62, 4.71 and 4.25) (Table 1, Fig. 2). CD26 is differentially expressed in many cell population but there have been no reports on the effect of porcine preimplantation embryos development. While we have no direct information about CD26 expression in the preimplantation embryos, blastocysts development in CD26 siRNA treated groups is significantly lower and the apoptosis is higher than control groups. It might be expected to be effected in porcine preimplantation embryo development. Loss of CD26 leading to high levels of bFGF in the nucleus that disrupts the normal cellular transcriptional program. Silencing of CD26 by siRNA induces G2-M arrest and cell death in prostate cancer cells. The morphology and growth pattern of cells transfected with CD26-specific siRNA reverted to malignant phenotype and

Table 1. Effect of cd26 siRNA injection on in vitro development of porcine embryos

Treatments	No. of IVC embryos	No. (%)* of embryos developed to		No. of total cells	No. (%) of apoptotic cells
		2-cell ≤	BL (7D)		
Uninjected	109	92 (84.4 ± 2.9)	46 (42.2 ± 11.9)	44.5 ± 11.4	1.2 (2.6 ± 1.5)
Control siRNA	94	79 (84.1 ± 2.9)	35 (37.2 ± 14.1)	48.6 ± 17.7	2.3 (4.7 ± 4.3)
CD26 siRNA	85	73 (85.9 ± 3.6)	9 (10.6 ± 12.4)*	35.3 ± 9.7	1.1 (4.2 ± 1.3)

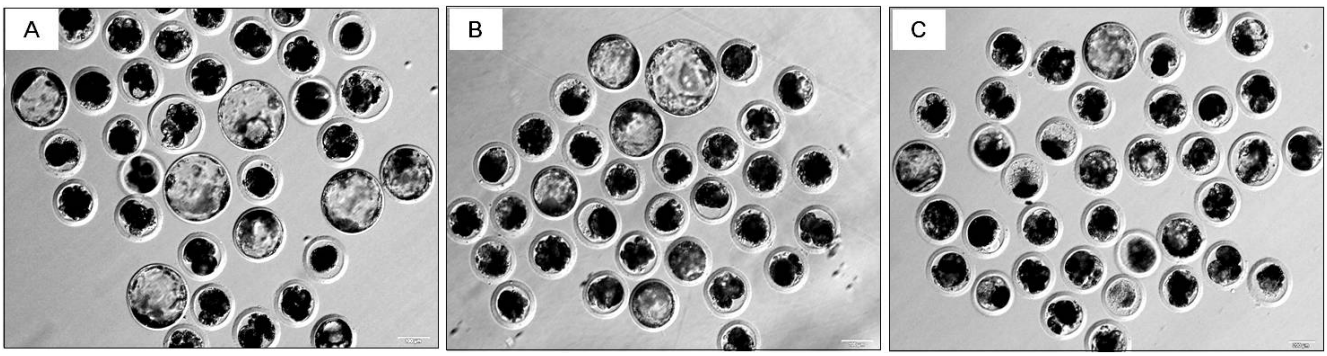

Fig. 1. Representative photographs showing the developmental morphology of porcine embryos obtained uninjected (A), the control siRNA (B) andCD26 siRNA (C) injection.

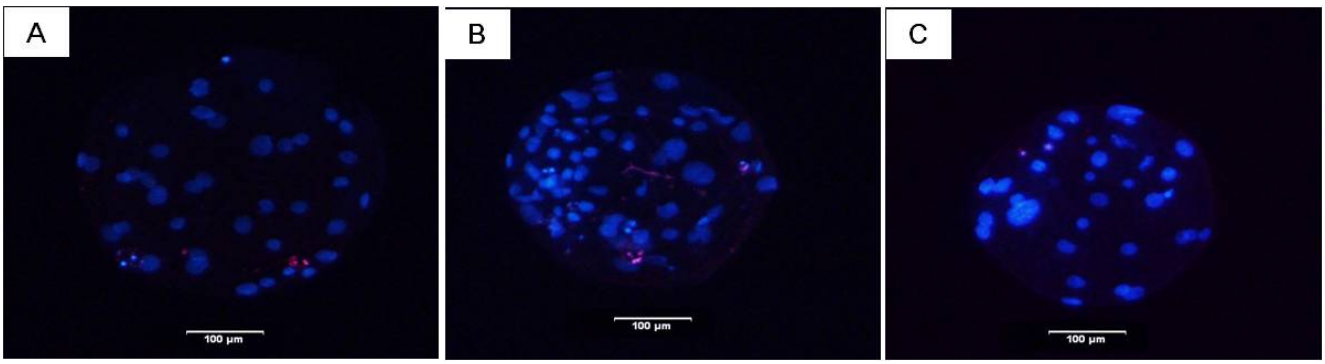

Fig. 2. Representative photographs porcine parthenogenetic blastocysts. The embryos labeled for Hoechest 33342 (blue). A; uninjected, B; control siRNA and C; CD26 siRNA

started to grow in disorganized colonies (Umadevi *et al*., 2013). The multifunctional activities of CD26 are dependent on cell type and intracellular or extracellular conditions that influence its role a proteolytic enzyme, cell surface receptor, cell adhesion and apoptosis. The results of the present study suggest that CD26 depletion is associated with decreased development rate in porcine parthenogenetic embryos. Further work is required to elucidate the molecular mechanisms of CD26 in porcine embryogenesis.

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