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Effects of Transcription Factor AP2γ on Gene Expression of Desmosome Components in Mouse Embryos

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ABSTRACT

Transcription factor called activating enhancer binding protein 2C (AP2-gamma) is found in a variety of species and expressed from oocyte stage onwards, particularly restricted to the trophectoderm. Recent studies demonstrated that ablation of Tfap2c led to failure of tight junction biogenesis, particularly the knock-down embryos of Tfap2c did not form cavity from morula to blastocyst in mouse and pig. We speculated that the Tfa2pc may also be involved in desmosome biogenesis because blastocoel formation is coincident with the establishment of desmosome. To determine this, we depleted Tfap2c injecting siRNA into one-cell zygote and analysed the expression levels of genes that are required for desmosome complex such as PkP2, Pkp3, Dsc2, and Dsg2. We found only Pkp3 was up-regulated in the knockdowned morula embryos. Interestingly, upstream region of Pkp3 had putative Tfap2c binding sites. In conclusion, our results suggest that Tfap2c is not a crucial factor but somehow it might be involved in desmosome biogenesis directly or indirectly via Pkp3.

(Key words : Tfap2c, Desmosome, Blastocoel, Cavitation, RNA interference)

INTRODUCTION

Transcription factor AP2γ (Tfap2c, also known as Tcfap2c) belongs to a family of DNA-binding transcription factor genes called activating enhancer binding protein 2 (AP2) found in a variety of species such as the human, mouse, and cattle (Aston *et al*., 2009; Eckert *et al*., 2005). The Tfap2 family are reported to be expressed from oocyte stage onwards, particularly restricted to the outer cells at the blastocyst stage. Recent studies demonstrated that Tfap2c is a core regulator of tight junction biogenesis and cell proliferation during the transition between morula to blastocyst in mouse and pig (Choi *et al*., 2013; Lee *et al*., 2015). However, it is not well elucidated whether Tfap2c is involved in other junctional biogenesis such as desmosome. A previous study reported that Cdh1 (E-cadherin), beta-catenin, and Par3 were not affected by Tfa2pc although these genes have AP-2C binding motif on their upstream region (Choi *et al*., 2012).

Desmosome first assemble in the morula or early blastocyst at the mouse trophectoderm (TE), concomitant with outer epithelial polarization and blastocyst cavitation. Tight junction is associated with actin filaments whilst desmosome is connected with intermediate filaments via plaque proteins such as plakophilin(Pkp), Plakoglobin(Pkg), and Junctinal plako globin(Jup) (Gallicano *et al*., 1998; Garrod and Chidgey, 2008). The molecular steps underlying the desmosome biogenesis in the preimplantation mouse embryos had been examined by immunocytochemistry(Fleming *et al*., 1991). The early study showed localisation of desmosomal proteins such as desmoplakins (Dsp), plakoglobin(Pkg), desmoglein(Dsg), and desmoclolin(Dsc) and reveal that onset of blstocolele formation is coincident with the desomosome establishment(Fleming *et al*., 1991). In addition, a loss of function study demonstrated that desmoplakin (Dsp) is essentially required as a bridge between intermediate filaments and desmosomal plaque proteins (Gallicano *et al*., 1998).

Given the important role of desomosomes and cavitation, we speculated that Tfap2c might regulate transmembrane protein and plaque protein expression at the transcriptional level because Tfap2c depleted embryos failed to form cavities(Choi *et al*., 2012).

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MATERIALS AND METHODS

Embryo Culture and Micromanipulation

As described previously (Choi *et al*., 2012), B6D2F1 female mice were injected intraperitoneal with 5 IU of PMSG and 5 IU of hCG 48 h later. The female mice displaying a copulation plug were mated with B6D2F1 male mice, and then the female mice were sacrificed at 16 hours post hCG by CO_2 inhalation, and the oviduct were excised in order to collect one-cell stage zygotes. The zygote showing two pronuclei were injected either 5-10 pL of 10 μM Tfap2c siRNA (siGenome, Dharmacon, Fafayette, CO, USA) or scramble siRNA as a control by using a PL100 picoinjector (Harvard Apparatus, Hollistan, MA USA). The injected embryos were cultured in modified KSOM medium (EMD Millopore, Billerica, MA, USA) under mineral oil at 37℃ in a humidified atmosphere of 5% CO_2. Animal care was in accordance with the institutional guidelines of Chungnam National University.

qRT-PCR and Analysis of Gene Expression

Embryos were washed in DPBS, snap frozen in liquid nitrogen and stored at −70℃. Total RNA isolated from a pool of embryos at morula or blastocyst stages by using the PicoPure RNA isolation kit (Arcurus, Mountain View, CA, USA) was used as templates for the first stand cDNA synthesis. SuperScript II reverse transcriptase and random primers/oligo dT (invitrogen, Carlsbad, CA, USA) were used to synthesis cDNA. qRT- PCR analysis was conducted utilizing gene-specific designed primers (SYBR Green detection), TaqMan probe (*Ubf*) and a StepOnePlus real-tiome PCR system (Applied Biosystems, Foster City, CA, USA). PCR was performed as follows: denaturation at 95℃ for 10 min, 40 cycles of amplification and quantification at 94℃ for 10 s, 55 or 60℃ for 30 s, and 72℃ for 30 s with a single fluorescence measurement, melting at 65-95℃ with a heating rate of 0.2℃/s, and continuous fluorescence measurement and cooling to 12℃. Fluorescence data were acquired after the extension step of PCR reactions. PCR products were analysed by generating melting curves to ensure gene-specific amplifications and the relative quantification of gene expression was determined by the $2^{-\Delta\Delta Ct}$method(Livak and Schmittgen, 2001). *Ubtf* and GFP mRNA were used as an endogenous and an exogenous control, respectively. PCR primer sequences are listed in Table 1.

Statistical Analysis

The qRT-PCR data were subjected to analysis of variance (ANOVA) combined with the Tukey post hoc test in statistical analysis software, GraphPad Prism 5(GraphPad software, CA, USA).). A *p*-value of <0.05 was considered statistically significant.

RESULTS

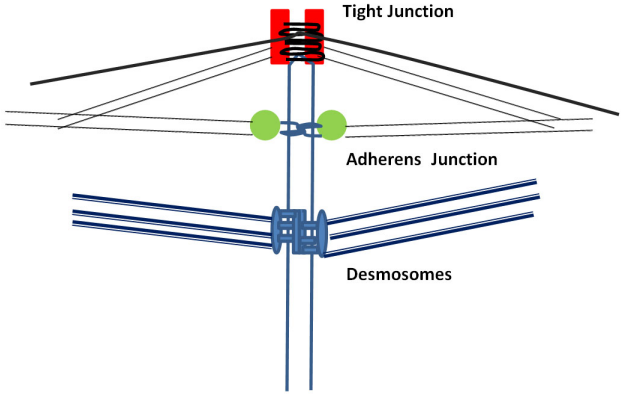
We first examined effectiveness of gene specific Tcfap2c siRNA using qRT-PCR. In this study we employed 10 μM of siRNA because use of 100 μM led to arrest at the morula stages. We observed about 60% of blastocyst development and 62% of Tfap2c knockdown. We then investigated gene expression of desmosome components such as plakophilin(Pkp), desomglein(Dsg), desomcollin (Dsc), and protein kinase C, zeta (Prkcz) in the tfap2c KD morula and blastocyst embryos.

In the Tfap2c KD morula embryos, Dsg2 was significantly down-regulated more than 2-fold ($p<0.05$) whilst Pkp was up-regulated more than 2.4-fold. However, Pkp2 and Dsc2 transcripts levels were not changed.

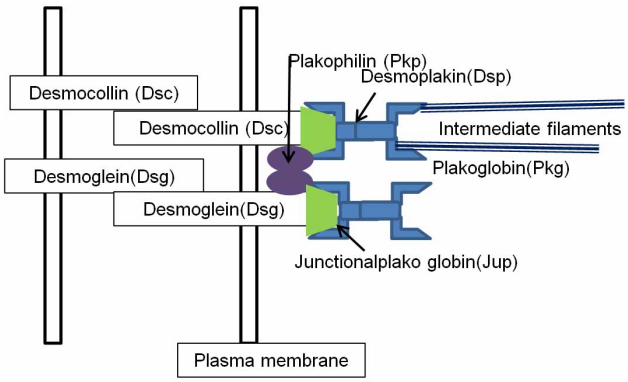
At the blastocyst stages, the KD embryos lost 57% of tfap2c mRNA. The ablation efficacy was similar to that in the morula KD embryos. However, gene expression patterns were different from those of the morula KD. All the examined genes such as Prkcz, PkP2, PkP3, Dsc2, and Dsg2 were not significantly different although Pkrcz, Pkp2, and Dsg2 were less expressed and Pkp3 were relatively higher in the KD blastocyst, but not significant ($p>0.05$).

Table 1. Primer sequences

Gene	Forward (5′→3′)	Reverse (5′→3′)
Prkcz	CAT TCA TGT TTT CCC AAG CA	TCG GTA CAG CTT CCT CCA TG
Pkp2	CCA ATG GCT TGC TTG ATT TT	CCT TCT CAT GAT CTT GGT CCC
Pkp3	ATT TTT GAG CTG TTG CGG AC	GTG GTC ACT GGA GGA CAG GT
Dsc2	GGG ACA CCT GTT GAC CCT T	CAA CAA ATT TCT GGG CAG GT
Dsg2	AGT GGG CTG TGA TAA CTG GC	GAA GGG TGA CAA TCC CTT CA



(A) Two adhesive epithelial junction such as adherens and desomsomes, and one tight junction are essential for maintenance of embryogenesis

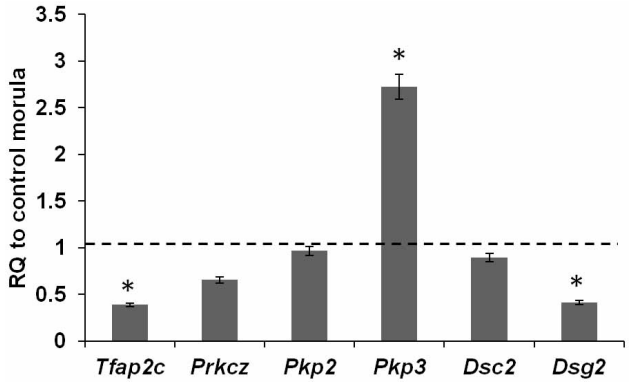


(B) Detail view of desmosome. Two types of transmembrane desomcollin, desomglein, plaque proteins including plakophilin, junctional plako globin, plakoglon, desmoplakin, and intermediate filaments such as keratin are required for the formation of desmosome

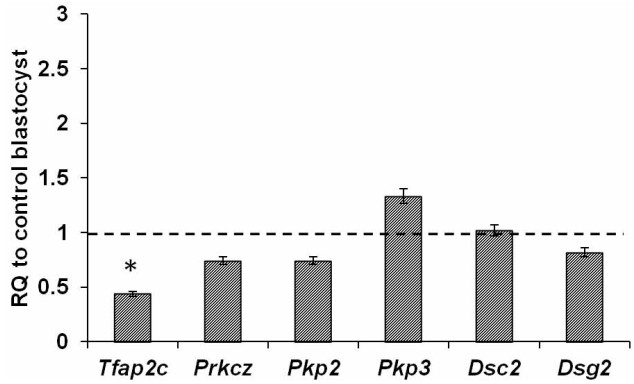
Fig. 1. Schematic view of epithelial junctional complex in mouse embryos.

DISCUSSION

Desmosomes are spotlike multi-molecular membrane complexes including transmembrane proteins, and plaque proteins, subsequently that are connected with intermediate filaments. Originally, desmosomes provide inter-cellular adhesion, intermediate filament anchorage in epithelial cells. As mentioned above, some proteins expression and localization involved in adheren and tight junction, for example *Cdh1*, *Ctnnb1* and *Par 3* were not affected by Tfap2c in mouse and pig (Choi *et al*., 2012; Lee *et al*., 2015). However, desomosomes were not investigated since the components and the biological function have been reported (Fleming *et al*., 1991; Gallicano *et al*., 1998).



(A) In the tfap2c KD morula, Pkp3 was upregulated but others were not affected



(B) After blastocyst formation with cavity, most desmosome related genes were not changed. * significantly different (p<0.05); RQ (relative quantification)

Fig. 2. Effects of Tfap2c knockdown on gene expression.

The timing of desmosome biogenesis seems to be regulated by the expression patterns of genes involved in cadhereins, suggesting that the cytoplasmic plaque proteins may crucial for the formation of desmosome, and particulary cavitation during the transition between morula and blastocyst. Thus, we focussed on plakophillin (Pkp2 and Pkp4) and transmembrane proteins (Dsc2 and Dsg2).

Interestingly, we found that Pkp3 were negatively regulated by Tfap2c, and 26 putative sites were predicted in upstream region of Pkp3 (−1,000 bp) analysed using Jaspar, suggesting that Tfap2c is involved in the expression of Pkp3. Although *in silico* analysis revealed that the potential interaction between the transcription factor, Tfap2c and Pkp3, we did not examined the direct interaction using ChIP (Chromatin Immunoprecipitation) or PLA (Proximal Ligation Assay) (Choi *et al*., 2013).

Here we report that Tfap2c is not a crucial factor but somehow it might be involved in desmosome biogenesis directly or indirectly via Pkp3.

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