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 trophoderm. Recent studies demonstrated that ablation of Tfp2c led to failure of tight junction biogenesis, particularly the knock-down embryos of Tfp2c 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 blastocoele formation is coincident with the establishment of desmosome. To determine this, we depleted Tfp2c by 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 knockeddown morula embryos. Interestingly, upstream region of Pkp3 had putative Tfp2c binding sites. In conclusion, our results suggest that Tfp2c is not a crucial factor but somehow it might be involved in desmosome biogenesis directly or indirectly via Pkp3.

(Key words : Tfp2c, Desmosome, Blastocoele, Cavitation, RNA interference)

INTRODUCTION

Transcription factor AP2γ (Tfp2c, 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 Tfp2 family are reported to be expressed from oocyte stage onwards, particularly restricted to the outer cells at the blastocyst stage. Recent studies demonstrated that Tfp2c 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 Tfp2c 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 Tfp2c 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 Junctional plakoglobin(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), desmogelin(Dsg), and desmocollin(Dsc) and reveal that onset of blastocoele formation is coincident with the desmosome 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 desmosomes and cavitation, we speculated that Tfp2c might regulate transmembrane protein and plaque protein expression at the transcriptional level because Tfp2c 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 CO₂ 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 Tiap2c siRNA (siGenome, Dharmacon, Fayetville, CO, USA) or scramble siRNA as a control by using a PL100 picoinjector (Harvard Apparatus, Hollistian, MA USA). The injected embryos were cultured in modified KSOM medium (EMD Millipore, Billerica, MA, USA) under mineral oil at 37°C in a humidified atmosphere of 5% CO₂. 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°C. 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 strand 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-time PCR system (Applied Biosystems, Foster City, CA, USA). PCR was performed as follows: denaturation at 95°C for 10 min, 40 cycles of amplification and quantification at 94°C for 10 s, 55 or 60°C for 30 s, and 72°C for 30 s with a single fluorescence measurement, melting at 65-95°C with a heating rate of 0.2°C/s, and continuous fluorescence measurement and cooling to 12°C. 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^ΔΔCt method (Livak and Schmittgen, 2001). Ubf 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 Tiap2c knockdown. We then investigated gene expression of desmosome components such as plakophilin(Pkp), desmoglein(Dsg), desmocollin (Dsc), and protein kinase C, zeta (Prkcz) in the Tiap2c KD morula and blastocyst embryos.

In the Tiap2c 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 tafap2c 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 Pkrkc, 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5’→3’)</th>
<th>Reverse (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prkcz</td>
<td>CAT TCA TGT TTT CCC AAG CA</td>
<td>TCG GTA CAG CTT CCT CCA TG</td>
</tr>
<tr>
<td>Pkp2</td>
<td>CCA ATG GCT TGC TGG ATT TT</td>
<td>CCT TCT CAT GAT CTT GGT CCC</td>
</tr>
<tr>
<td>Pkp3</td>
<td>ATT TTT GAG CTG TGG CGG AC</td>
<td>GTG GTC ACT GGA GGA CAG GT</td>
</tr>
<tr>
<td>Dsc2</td>
<td>GGG ACA CCT GTT GAC CCT T</td>
<td>CAA CAA ATT TCT GGG CAG GT</td>
</tr>
<tr>
<td>Dsg2</td>
<td>AGT GGG CTG TGA TAA CTG GC</td>
<td>GAA GGG TGA CAA TCC CTT CA</td>
</tr>
</tbody>
</table>
**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 Cadherin1, Cdh1 and Par 3 were not affected by Tfap2c in mouse and pig (Choi et al., 2012; Lee et al., 2015). However, desmosomes were not investigated since the components and the biological function have been reported (Fleming et al., 1991; Gallicano et al., 1998).

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 cadherins, 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 plakophilin (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.
ACKNOWLEDGEMENT

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REFERENCES


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