Biochemistry and Biophysics Reports 15 (2018) 13–18

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

The epithelial-mesenchymal transition induced by transcription factor LEF-1 is independent of β-catenin

Wakako Kobayashi, Masayuki Ozawa*

Department of Biochemistry and Molecular Biology, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan

ARTICLE INFO

Keywords:
EMT
LEF-1
β-catenin
CRISPR/Cas9
Knockout

ABSTRACT

Transcription factor lymphoid-enhancer–binding factor 1 (LEF-1) is a key molecule in the Wnt/β-catenin signaling pathway. *Slug* is one of the Wnt/β-catenin target genes and can induce epithelial–mesenchymal transition (EMT). Previously, we have shown that not only wild-type LEF-1 but also LEF-1 lacking the amino-terminal β-catenin–binding region can induce EMT, suggesting that LEF-1 acts independently of β-catenin. Because it has been reported that LEF-1 interacts with β-catenin outside the amino-terminal domain, namely, in the middle part of the molecule, the possible participation of β-catenin has not been formally ruled out. To determine the involvement of β-catenin in the LEF-1–induced EMT, we produced MDCK cells with a deletion of the β-catenin gene and then expressed LEF-1 in the cells. We found that LEF-1 induced EMT in those cells. In the absence of β-catenin, γ-catenin has been shown to take over the role of β-catenin. To examine this possibility, we first established MDCK cells with a double knockout of β-catenin and γ-catenin genes and then expressed LEF-1 in these cells. We found that LEF-1 can induce EMT in these cells; therefore, we conclude that neither β-catenin nor γ-catenin expression is necessary for the LEF-1–mediated induction of EMT.

1. Introduction

Epithelial–mesenchymal transition (EMT) is known as one of the essential steps for tissue remodeling, organ development, wound healing, and cancer metastasis [1–4]. When EMT is induced, epithelial characteristics are lost and mesenchymal properties are acquired. EMT-related transcription factors—Snail, Slug, and ZEB1/2—suppress the expression of epithelial markers such as E-cadherin, while increasing expression of mesenchymal markers N-cadherin and fibronectin and augmenting cell motility and the invasive potential.

Members of the lymphoid-enhancer–binding factor 1/T-cell factor (LEF-1/TCF) family are key transcription factors that interact with β-catenin and activate Wnt/β-catenin signaling [5]. They regulate the cell cycle–related gene cyclin D1, cell growth–related gene *c-myc*, and EMT-related gene *SNAI2 (Slug)* [6,7]. On the other hand, there are some reports that LEF-1 can act without interacting with β-catenin in lung adenocarcinomas, small B-cell lymphomas, and sebaceous skin tumors [8–10]. Expression of LEF-1 lacking the amino-terminal β-catenin–binding domain (ΔNLEF-1) leads to sebaceous skin tumors in mice [11]. In fact, we found that the expression of ΔNLEF-1 induces EMT in MDCK cells [12]. These observations have suggested that β-catenin is not necessary for the LEF-1–mediated induction of EMT. Nonetheless, there is a report that in addition to the well-established amino-terminal β-catenin–binding domain of LEF-1, β-catenin binds to another site of LEF-1: residues 150–175 [13]. Because of these findings, the possibility of involvement of β-catenin in EMT induction is a contentious topic.

It is known that γ-catenin (also known as plakoglobin) is a scaffold protein at the adherens junction and in the desmosome [14,15] and is highly homologous to β-catenin. Disruption of the β-catenin gene in F9 cells (via a gene knockout; KO) does not affect cell–cell adhesion and cell morphology [17]. Additional inactivation of the γ-catenin gene in these cells disrupts cell–cell adhesion and induces morphological alterations [17]. E-cadherin expression is downregulated and cytoplasmic aggregates of E-cadherin are frequently observed [17]. In Wnt signaling cascades, γ-catenin can act as an alternative to β-catenin. Indeed, in β-catenin–deficient cells, γ-catenin, instead of β-catenin, plays a key role in the transcriptional activity of *TCF* and *LEF-1* [16].

To determine the involvement of β-catenin in LEF-1–induced EMT, we established MDCK cells with disruption of the β-catenin gene using the CRISPR/Cas9 gene editing system and then introduced a LEF-1 expression vector. We found that expression of LEF-1 induces EMT in these cells. We also generated MDCK cells in which β-catenin and γ-

Abbreviations: EMT, epithelial–mesenchymal transition; LEF-1, lymphoid-enhancer–binding factor 1; TCF, T-cell factor; KO, knockout; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR-associated 9; PAM, protospacer-adjacent motif; DKO, double knockout

* Corresponding author.
E-mail address: mozawa@m.kufm.kagoshima-u.ac.jp (M. Ozawa).

https://doi.org/10.1016/j.bbrep.2018.06.003
Received 27 April 2018; Received in revised form 4 June 2018; Accepted 7 June 2018

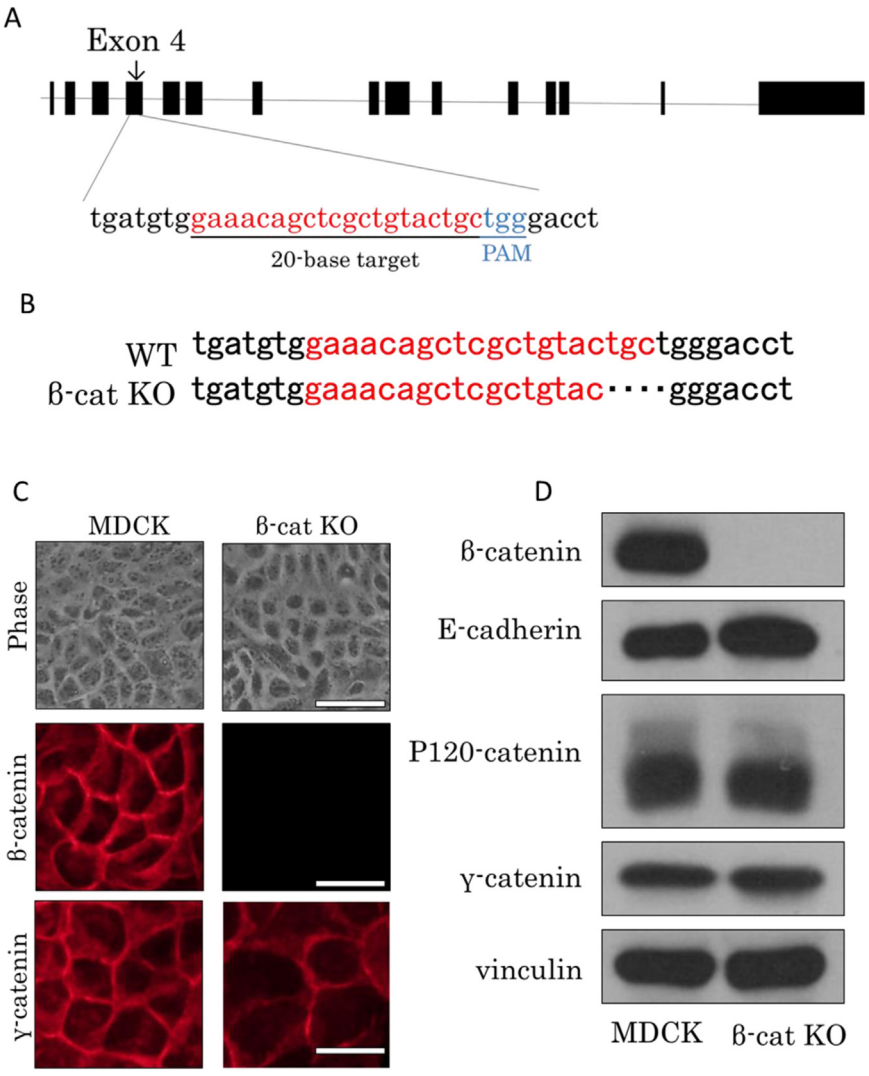

Fig. 1. The knockout of the β-catenin gene in MDCK cells. (A) Schematic representation and a position of the candidate gRNA target site in exon 4 of the β-catenin gene (*CTNNB1*). Targeted and PAM sequences are red and blue, respectively. (B) Genome sequence analysis. Four-nucleotide sequences are deleted in β-catenin knockout cells. At least eight genome sequences of cell clones were analyzed, and all clones yielded the same results. (C) Cell morphology and immunofluorescent staining. Phase contrast microscopy shows that β-cat KO cells have no morphological changes as compared with parental MDCK cells. Immunofluorescent staining using the anti–β-catenin antibody showed that β-catenin was undetectable in β-cat KO cells, but γ-catenin was still present at the plasma membrane. Scale bar, 50 μm. (D) Immunoblot analysis of β-catenin, E-cadherin, p120-catenin, and γ-catenin in MDCK cells and β-cat KO cells. Vinculin served as a loading control.

catenin genes were disrupted to rule out the possible compensation of β-catenin by γ-catenin. We found that LEF-1 induces EMT in these cells. These results revealed that LEF-1 induces EMT independently of β-catenin and γ-catenin.

2. Materials and methods

2.1. Plasmids and guide RNA (gRNA) synthesis

Mouse LEF-1 cDNA was kindly provided by Rolf Kemler (Max Planck Institute for Immunobiology, Germany). HA-tagged LEF-1 expression vector (pCAGGS-LEFHA) has been described previously [12]. A CRISPR/Cas9 knockout vector (pCGSapI) was kindly provided by Takayuki Sakurai (University of Sinshu, Japan). The pCGSapI vector includes human Cas9 cDNA under control of the CAG promoter and expresses gRNA via the U6 promoter [18]. The pCGSapI vector was digested with the SapI enzyme. To construct the KO vectors for β-catenin and γ-catenin, we selected a 20-base sequence upstream of the PAM site (NGG) for β-catenin in exon 4 of the canine *CTNNB1* gene, and for γ-catenin, in exon 3 of the canine *JUP* gene. The sequences of oligonucleotides were as follows: β-catenin gRNA, 5′-ACCGGAAACAGCT CGCTGTACTGCG-3′ and 5′-AAACGCAGTACAGCGAGCTGTTTCC-3′; γ-catenin gRNA, 5′-ACCGCACCAAACTGCTCAACGACGG-3′ and 5′-AAAC CGTCGTTGAGCAGTTTGGTGC-3′. Synthesized oligonucleotides for target KO gRNA were annealed and cloned at SapI sites into the pCGSapI vector, which was then transfected into JM109 cells. Clones were purified and subjected to DNA sequence analysis to check the oligonucleotide insert (Eurofins, Japan). Selection markers for cell transfection were neomycin (G418), hygromycin, and blasticidin resistance genes.

2.2. Cell transfection

MDCK cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% of fetal calf serum. The cells were transfected with 10 μg of the expression vectors or KO vectors by the calcium phosphate method [19]. To construct the β-catenin KO MDCK cells (termed β-cat KO cells), MDCK cells were cotransfected with pCGSapI-β-catenin gRNA and the blasticidin resistance gene. After 48 h of transfection, the cells were subjected to selection with blasticidin S (8 μg/ml). Single colonies were isolated and analyzed by immunofluorescent staining and immunoblotting with an anti–β-catenin antibody. To prepare cells with a double KO (DKO) of β-catenin and γ-catenin (termed βγ-DKO cells), β-cat KO cells were cotransfected with pCGSapI-γ-catenin gRNA and the hygromycin resistance gene. After selection with hygromycin B (300 μg/ml), single colonies were isolated and analyzed by immunofluorescent staining and immunoblotting with an anti–γ-catenin antibody. To obtain LEF-1–overexpressing cells, β-cat KO and/or βγ-DKO cells were transfected with the LEF-1 expression vector. After selection with G418, single colonies were isolated and