β-Catenin mediates adriamycin-induced albuminuria and podocyte injury in adult mouse kidneys

Eija Heikkilä1,*, Juuso Juhila1,2,*, Markus Lassila1, Marcel Messing1, Nina Perälä3, Eero Lehtonen4,5, Sanna Lehtonen1,4, Joseph Sjef Verbeek6 and Harry Holthofer1,2

1Haartman Institute, Department of Bacteriology and Immunology, University of Helsinki, Finland, 2Centre for BioAnalytical Sciences, Dublin City University, Ireland, 3Institute of Biomedicine, Medical Biochemistry and Developmental Biology, Biomedicum, 4Haartman Institute, Department of Pathology, 5Experimental Animal Centre, University of Helsinki, Finland and 6Department of Human Genetics, Leiden University Medical Center, The Netherlands

Correspondence and offprint requests to: Harry Holthofer. E-mail: harry.holthofer@dcu.ie

*The first two authors contributed equally to this study.

Abstract

Background. Glomerular slit diaphragm (SD) presents a modified adherens junction composed of molecules belonging to both immunoglobulin and cadherin superfamilies. Cadherins associate with the cytosolic scaffolding protein β-catenin, but the precise role of β-catenin in mature or injured podocytes is not known.

Methods. The conditional podocyte-specific β-catenin-deficient mouse line was generated using the doxycycline-inducible Cre-loxP system. Expression of the β-catenin-deficient gene was turned off at the age of 8 weeks by doxycycline treatment and the kidney phenotype was analysed. In addition, β-catenin-deficient and control mice were treated with adriamycin (ADR) and analysed for albuminuria and morphological alterations.

Results. Deletion of β-catenin in mature podocytes did not change the morphology of podocytes nor did it lead to albuminuria. However, lack of β-catenin attenuated albuminuria after ADR treatment. Electron microscopic examination showed increased podocyte foot processes expansion associated with SD abnormalities in ADR-treated control mice compared to β-catenin-deficient mice.

Conclusions. These results show that β-catenin in podocytes is dispensable for adult mice, but appears to be important in modulating the SD during ADR-induced perturbation of the filtration barrier.

Keywords: albuminuria; β-catenin; inducible mouse models; slit diaphragm

Introduction

The glomerular filtration barrier consists of fenestrated capillary endothelium, layered glomerular basement membrane (GBM) and glomerular visceral epithelial cells or podocytes. Podocytes cover the GBM from outside and present with highly ordered cellular extensions, foot processes, linked to each other by a unique intervening structure, the slit diaphragm (SD) [1]. The SD is crucial in regulating the passage of circulating plasma proteins into primary urine. Glomerular diseases with albuminuria are characterized by morphological changes of the podocytes, including loss of SDs and foot process effacement. The degree of these changes is associated with the severity of albuminuria [2].

The SD is a unique cell–cell contact with characteristics of both adherens [3] and tight junctions [4] and is composed of proteins including P-cadherin, zonula occludens-1 (ZO-1) and immunoglobulin superfamily proteins, nephrin [5] and Neph1–3 [6–10]. Nephrin is a well-established backbone of the SD and associates, among other known components, with P-cadherin [5,11]. β-Catenin links this classical cadherin to the actin cytoskeleton via α-catenin, which is essential for the proper adhesion activity of cadherins [12]. However, it is not known if β-catenin is important for maintaining SDs.

The expression of β-catenin in mature podocytes has been controversial, since some authors have reported that it is only present in immature podocytes [13,14], whereas others have localized β-catenin in mature podocytes [15]. The discrepancy may be due to differences in the sensitivities of the antibodies used in the studies, but it also indicates that β-catenin expression is apparently reduced during podocyte maturation. Podocyte injury leads to replacement of SDs with junctions which resemble intercellular contacts during podocyte differentiation. Therefore, we hypothesized that β-catenin may either play a role in the SD integrity or participate in modulation of SD during podocyte injury.

Conventional β-catenin knockout (KO) mice are not suitable to explore the role of β-catenin in the adult, fully functional kidney, because these mice die during embryonic development [16–18]. Therefore, we used the podocyte-specific doxycycline-inducible Cre recombinase mouse.
line [19] and a mouse line containing a floxed β-catenin gene [18] to remove β-catenin expression specifically in mature podocytes. After induction of the deletion of the β-catenin gene by doxycycline treatment at the age of 8 weeks, the mice maintained normal kidney phenotype. However, when albuminuria was induced by adipymycin (ADR) treatment, the induced β-catenin deficiency protected the mice from development of podocyte injury and albuminuria.

Materials and methods

Ethics

All animal care conformed to the European Communities Council Directive 86/609/EEC, and the experiments had approval of the Experimental Animal Committee of the University of Helsinki and the Provincial Government of Southern Finland.

Generation of β-catenin-deficient mice

Generation of the transgenic mouse line expressing doxycycline-inducible Cre recombinase under the podocin promoter (IRC-cre) has been reported earlier in detail [19]. The IRC-CRE (β-cattotox/fl) mice were cross-bred with a mouse line with the loxP sites inserted into β-catenin gene (β-cattfl/fl) (B6.129-Catnb tm2Kem/J; Jackson Laboratories, Bar Harbor, ME). The resulting offspring (β-cattfl/fl/Cr) were then intercrossed to create the β-catenin-deficient (β-cattfl/fl/Cr) mouse line in which β-catenin could be switched off by doxycycline administration as described in detail earlier [19]. The IRC-CRE mouse line was crossed for five generations to the C57Bl/6J mouse strain and β-catenin-floxed littermates were used for more than 10 generations in the same background. Littermate controls were used in all experiments.

The offspring were identified by PCR on tail genomic DNA (50–100 ng) with 35 cycles of 95°C (30 s), 54°C (30 s) and 72°C (30 s) and a final extension of 72°C for 5 min and analysed with standard agarose gel electrophoresis (1.5% gel) [19]. To detect the β-floxed allele, sense primer RM41 (5′-aaggtagagtgatgaaagttgtt-3′) and antisense primer RM42 (5′-caccaggctgcctctattc-3′) were used. These primers generate 324 and 221 bp products from the floxed and wild-type alleles, respectively [18]. For detection of Cre transgene CreSense and CreAntisense, 5′-cattgtcctctgtctattc-3′ sense primer and CreAntisense primer RM41 (5′-aaggtagagtgatgaaagttgtt-3′) and antisense primer RM42 (5′-caccaggctgcctctattc-3′) were used. These primers generate 324 and 221 bp products from the floxed and wild-type alleles, respectively [18]. For detection of Cre transgene CreSense and CreAntisense, 5′-cattgtcctctgtctattc-3′ sense primer and CreAntisense primer RM41 (5′-aaggtagagtgatgaaagttgtt-3′) and antisense primer RM42 (5′-caccaggctgcctctattc-3′) were used. These primers generate 324 and 221 bp products from the floxed and wild-type alleles, respectively [18].

When the offspring reached the age of 8 weeks, Cre recombinase was activated by administration of 1.0 mg/ml doxycycline in drinking water including 5% sucrose for 2 weeks. The doxycycline solution was changed activated by administration of 1.0 mg/ml doxycycline in drinking water including 5% sucrose for 2 weeks. The doxycycline solution was changed activated by administration of 1.0 mg/ml doxycycline in drinking water including 5% sucrose for 2 weeks. The doxycycline solution was changed

Expression Analyses

Mice were sacrificed by cervical dislocation, tissues were immediately dissected and either fixed in 4% paraformaldehyde (PFA) or snap frozen in liquid nitrogen. Preparation of sections for immunohistochemistry and immunofluorescence was performed as described earlier [19,20].

Immunohistochemistry. Sections (3–4 μm) were deparaffinized in xylene three times for 10 min and rehydrated through a series of ethanol (100 to 50%, 3 min each) [20]. Antigen retrieval was performed by heating the sections in 10 mM citrate buffer (pH 6) in microwave oven three times for 5 min, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide in PBS (for β-catenin and P-cadherin stainings) or methanol (for β-catenin and CD2AP serial section stainings) for 20 min. In stainings with mouse monoclonal P-cadherin antibody (Invitrogen, Carlsbad, CA), the non-specific binding was blocked with HistoMouseTM-Max BeatTM blocking method (Invitrogen) according to manufacturer’s instruction. Rabbit polyclonal anti-β-catenin antibody (Santa Cruz Biotechnology, Santa Cruz, CA) stainings, the sections were blocked with 2% fetal bovine serum, 2% bovine serum albumin and 0.2% gelatine in PBS in 1 h at room temperature (RT). Serial sections stained with β-catenin (Santa Cruz) and CD2AP [21] rabbit polyclonal antibodies were blocked with GAS-BLOCK (Zymed Laboratories, San Francisco, CA) for 30 min. Primary antibodies incubations were performed at +4°C followed by PBS washes. Sections for β-catenin staining were incubated with Envision, peroxidase-conjugated anti-rabbit antibodies (Dako Cytomation, Carpinteria, CA) for 1 h. For P-cadherin, tyramine signal amplification system was used in combination with biotinylated secondary antibody and streptavidin-enzyme conjugate from Histostain SP kit (Invitrogen). Vectastain Elite ABC peroxidase system (Vector Laboratories, Burlingame, CA) was used for β-catenin and CD2AP serial section stainings according to manufacturer’s instructions. The peroxidase reaction for all antibodies was developed with AEC substrate (Dako) for 12 min. Finally, the sections were counterstained with haematoxylin for 1 min, mounted with Immuno-mount (Shandon, Pittsburgh, PA) and examined by Olympus AX70 microscope using a ×63 objective (Olympus, Tokyo, Japan).

Immunofluorescence microscopy. Cryosections (6 μm) were fixed with 2% PFA for 30 min at RT followed by permeabilization with 0.1% Triton X-100 in PBS, or alternatively, sections were fixed with ice-cold acetone for 10 min. Sections were blocked as described above for β-catenin staining and incubated with rabbit polyclonal antibodies against podocin (Sigma-Aldrich, St. Louis, MO), ZO-1 (Zymed) and intracellular part of rat nephrin [22] diluted in the blocking solution on/off at +4°C. Primary antibodies were visualized with TRITC-conjugated anti-rabbit antibody (Jackson Laboratories). All the specimens were viewed with Zeiss Axiopt® 2 microscope using a ×63 Plan-Neofluar objective (Carl Zeiss Jena GmbH, Jena, Germany).

Immunoblotting. Mouse kidney cortices or E12 mouse embryos were lysed using a glass homogenizer in RIPA-buffer (150 mM NaCl, 1% NP40, 0.5% deoxycholic acid, 0.1% sodium dodecyl sulphate (SDS), 50 mM Tris–HCl pH 8, 0.02% NaN3) supplemented with Complete, Mini, EDTA-free proteinase inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany) on ice. Insoluble material was removed by centrifugation at 15 000 g at +4°C for 15 min, and total protein concentrations were measured. Samples (100 μg for SD markers and 80 μg for Wnt signalling markers) were resolved by 10% SDS–polyacrylamide gel electrophoresis, transferred to Immobilon–FL polyvinylidene fluoride (Millipore, Billerica, MA) and blocked with 5% skimmed milk in PBS at RT for 1 h. Membranes were incubated at +4°C overnight with primary antibodies diluted in 1% skimmed milk in PBS supplemented with 0.1% Tween-20. The following primary antibodies were used: rabbit anti-podo- cin (Sigma), rabbit anti-nephrin [22], rabbit phospho-glycogen synthase kinase 3 (GSK-3) (Cell Signalling Technology, Beverly, MA), mouse anti-active-β-catenin (Millipore) and mouse anti-a-tubulin (Sigma). The following day, membranes were washed with 0.2% Tween-20 in PBS followed by an hour incubation with anti-rabbit Alexa Fluor 680 (Molecular Probes, Eugene, OR) or anti-mouse Alexa Fluor 800 diluted in 1% skimmed milk in PBS supplemented with 0.1% Tween-20 and 0.01% SDS. After repeated washes with PBS containing 0.2% Tween-20, the signal was detected using the Odyssey infrared imaging system (LI-COR Biosciences GmbH, Bad Homburg, Germany).

Assessment of structural changes in glomeruli

Haematoxylin–eosin. The dissected kidneys were fixed in 4% PFA and embedded in paraffin. Sections (4 μm) were deparaffinized, stained with haematoxylin–eosin using standard procedures [23] and examined by Olympus AX70 microscope using ×20 and ×40 objectives (Olympus). Electron microscopy. For electron microscopic examination, kidney cortices were fixed in 1.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4) at RT for 2 h. Samples were postfixed in 1% osmium tetroxide (OsO4) in the same buffer for 1 h and stained en-bloc in 1% uranyl acetate in 10% ethanol for 1h, dehydrated in ethanol and embedded in LX112. Thin sections were stained with uranyl acetate and lead citrate and examined in a JEM-1400 Transmission Electron Microscope (Jeol) with orada side-mounted TEM CCD Camera (Olympus Soft Imaging Solutions GmbH).
Podocyte foot process effacement was measured from two mice of each genotype after ADR treatment using electron micrographs and ImageJ-program calibrated by the marker bar. The length of five random capillaries of four to five randomly selected glomeruli per mouse was measured. The number of podocyte foot processes was counted manually and expressed as the number of foot processes per micrometre GBM length. Student t-test was used for statistical analysis.

**Results**

**Creation of β-catenin-deficient mice**

To explore the role of β-catenin in adult mouse podocytes, a conditional β-catenin deficient mouse line was generated. Breeding of JRC-CRE transgenic mice expressing doxycycline-inducible Cre recombinase under the podocyte-specific podocin promoter [19] with mice with the floxed β-catenin gene [18] resulted in offspring in which β-catenin could be switched off specifically in podocytes by doxycycline administration (β-catfl/fl/Cre).

The doxycycline-induced podocyte-specific Cre-mediated recombination was confirmed by PCR analysis of DNA samples from different organs using primers specific for the recombinated allele [18]. The disappearance of β-catenin DNA fragment (exons 2–6) between two loxp sites was detected only in DNA samples from kidneys of β-catfl/fl/Cre mice after 2 weeks of doxycycline treatment at the age of 10 weeks (Figure 1).

Immunohistochemical stainings showed a very low level of β-catenin in glomeruli of β-catfl/fl/Cre control mice as well as in wild-type mice whereas tubules (data not shown) and glomeruli (Figure 6) of ADR-treated control mice expressed β-catenin. Due to the high abundance of β-catenin in tubules and a low signal in normal glomeruli, real-time PCR or immunoblotting assays could not be used to show quantitative differences after β-catenin deletion at RNA or protein levels in podocytes.

**Phenotype of the β-catenin-deficient mice**

Loss of β-catenin in podocytes at the adult age did not lead to noticeable changes in the general appearance, weight or behaviour of the mice within 2–6 weeks after induction of β-catenin deletion. These mice did not show albuminuria (data not shown) or any appreciable changes in the kidney morphology based on haematoxylin–eosin staining and light microscopy examination (Figure 2). Furthermore, no alterations in the expression level and localization of SD proteins including podocin, P-cadherin, ZO-1 and nephrin were observed by immunofluorescence or immunohistochemistry in β-catenin-deficient mice compared to controls (Figure 3A–H). Quantitative immunoblot analysis further confirmed that podocin and nephrin levels stayed similar in β-catenin-deficient and control mouse kidneys (Figure 3I).

**β-catenin deficiency in mature podocytes attenuates ADR-induced albuminuria**

To explore whether β-catenin contributes to podocyte injury and development of albuminuria, the nephrotoxic effects of ADR were studied after induced β-catenin deletion in the adult mice kidneys. ADR-induced nephropathy in mice is characterized by foot process effacement and albuminuria [24]. As expected, 3 days after ADR treatment albuminuria was significantly (P < 0.05) increased in the control β-catfl/fl/Cre mice and remained significant 6 days after the treatment compared to the albuminuria level before the treatment (Figure 4A). In contrast, the development of albuminuria was attenuated in the β-catfl/fl/Cre mice and reached no statistical significance compared to the level of albumin in urine before the ADR administration. Statistical significance was not found between the genotypes most likely due to the high standard deviation especially in β-catfl/fl/Cre mice (Figure 4A).
The slower development of albuminuria correlated with better general well-being score of ADR-treated \(\text{\textbeta-\textcatfl/fl/Cre}\) mice compared to \(\text{\textbeta-\textcatwt/wt/Cre}\) control mice (Figure 4B). The condition of \(\text{\textbeta-\textcatfl/fl/Cre}\) mice stayed close to normal during the whole ADR treatment, whereas the condition of \(\text{\textbeta-\textcatwt/wt/Cre}\) control mice got worse already 3 days after ADR treatment and remained significantly different between the genotypes during the whole experiment (Figure 4B).

**\(\text{\textbeta-\textcatenin}\)-deficiency in adult podocytes protects from ADR-induced foot process effacement**

Electron microscopic investigations of \(\text{\textbeta-\textcatwt/wt/Cre}\) kidneys 6 days after ADR administration showed podocyte foot process effacement associated with disruption and dislocation of the SDs towards the apical aspect of podocytes as well as focal GBM thickening. The alterations were not uniform and podocytes with normal morphology with intact SDs could also be found (Figure 5A–B). Podocyte foot processes were mostly preserved in \(\text{\textbeta-\textcatfl/fl/Cre}\) mice, although effaced foot processes could be detected in few areas. Similar focal GBM thickenings as in \(\text{\textbeta-\textcatwt/wt/Cre}\) were also occasionally found (Figure 5C–D). The quantification of podocyte foot process effacement showed that the number of podocyte foot processes per micrometre of GBM decreased significantly \((P \leq 0.001)\) from 1.7 ± 0.3 in \(\text{\textbeta-\textcatfl/fl/Cre}\) mice to 1.3 ± 0.2 in \(\text{\textbeta-\textcatwt/wt/Cre}\) (Figure 5E).

Immunohistochemical staining of kidneys 6 days after ADR administration showed podocyte-like staining for \(\text{\textbeta-\textcatenin}\) only in the \(\text{\textbeta-\textcatwt/wt/Cre}\) control mouse glomeruli (Figure 6A). The glomerular staining of \(\text{\textbeta-\textcatenin}\) in \(\text{\textbeta-\textcatenin}\)-deficient mice after ADR treatment (Figure 6B) apparently originates from endothelial and/or mesangial cells known to express catenins [13]. The control stainings omitting the primary antibody were negative (Figure 6C). To further confirm that \(\text{\textbeta-\textcatenin}\) is expressed in podocytes, we stained serial sections of \(\text{\textbeta-\textcatwt/wt/Cre}\) control mouse kidneys with \(\text{\textbeta-\textcatenin}\) and CD2AP, a marker for podocytes. Stainings confirmed that \(\text{\textbeta-\textcatenin}\) is expressed in podocytes (Figure 6D–G).

Wnt signalling activation leads to dephosphorylation and translocation of \(\text{\textbeta-\textcatenin}\) to the nucleus [27]. No nuclear localization of \(\text{\textbeta-\textcatenin}\) was detected after ADR treatment indicating that Wnt signalling was not induced. This was further confirmed by quantification of Wnt signalling markers, dephosphorylated active-\(\text{\textbeta-\textcatenin}\) and phospho-GSK-3\(\beta\) levels by immunoblot analysis. The results showed that the protein level of active-\(\text{\textbeta-\textcatenin}\) was similar between \(\text{\textbeta-\textcatwt/wt/Cre}\) and \(\text{\textbeta-\textcatfl/fl/Cre}\) mice kidneys. In addition, no expression of phospho-GSK-3\(\beta\) was found in \(\text{\textbeta-\textcatwt/wt/Cre}\) control and \(\text{\textbeta-\textcatfl/fl/Cre}\) mice kidneys further confirming that Wnt signalling is not activated after ADR treatment (Figure 6H). E12 mouse embryo lysate was used as a positive control for active-\(\text{\textbeta-\textcatenin}\) and phospho-GSK-3\(\beta\). These findings further indicate that the Wnt signalling cascade may not be induced by ADR treatment.

Since \(\text{\textbeta-\textcatenin}\) depletion in adult podocytes is protective against ADR-induced SD injury, the expression and/or lo-
calization of key proteins for SD integrity was assessed in the mice of different genotypes 6 days after ADR administration. By immunofluorescence microscopy, no obvious changes in the expression or localization of podocin (Figure 7A and B), nephrin and ZO-1 (data not shown) were detected. Immunohistochemical stainings of P-cadherin showed also showed a similar staining pattern in both genotypes (Figure 7C and D). In addition, quantitative immunoblot analysis of nephrin and podocin did not reveal statistically significant differences between β-catenin−/+/Cre and β-catenin−/−/Cre mouse kidneys (Figure 7E).

### Discussion

β-Catenin is an essential component of adherens junctions and is widely expressed in various cell types, but its role in podocytes in maintaining the structure and function of SD is unknown [3,15,28]. In the present study, we addressed this question by developing a mouse model in which we conditionally deleted β-catenin gene in the adult mouse podocytes using a doxycycline-inducible, podocyte-specific Cre recombinase mouse line [19]. Our key findings indicate that β-catenin appears to be morphologically and functionally dispensable for the normal adult podocyte while β-catenin-dependent pathways were shown to be vital for the pathogenesis of albuminuria and morphologic podocyte injury in the ADR model.

SD is a modified adherens junction containing members of, e.g. immunoglobulin (nephrin and NEPH1–3) [5–10] and cadherin (P- and VE-cadherin, FAT), superfamily proteins [3,29,30]. To function properly in cell adhesion, P- and VE-cadherin need to bind to β-catenin [31]. Furthermore, the indispensable SD protein nephrin has been shown to form a complex with P-cadherin [3,11]. Therefore, it may be assumed that loss of β-catenin could have an effect on the integrity of SD. However, our study showed that podocyte-specific deletion of β-catenin at the age of 8 weeks resulted in normal phenotype with well-preserved podocyte morphology and, importantly, the transgenic mice were normoalbuminuric. Similarly, recent data showed that deletion of β-catenin from podocytes during differentiation at the capillary loop stage did not influence podocyte maturation and SD formation [32]. The results imply that β-catenin-mediated pathways are dispensable both at later stages of podocyte differentiation and maintenance of the SD in the mature podocyte. The function of β-catenin may be compensated by a homologous protein γ-catenin which has been shown to take over the role of β-catenin in other cell types [17,32–34], or by other yet unidentified compensating components.

Since β-catenin is not essential for maintaining the SD, we investigated whether it plays a role in the development of podocyte injury and proteinuria. To this end, we treated β-catenin-deficient and control mice with ADR, which has been shown to cause podocyte injury in mice [24]. Notably, the development of ADR-induced albuminuria was attenuated in mice deficient of β-catenin. Furthermore, β-catenin−/−/Cre mice showed increased ADR-induced podocyte foot process effacement, disruption of the SD structures compared to β-catenin−/+/Cre indicating that β-catenin mediates ADR-induced podocyte injury. Also Dai et al. recently showed that lack of β-catenin protects against ADR-induced albuminuria and podocyte injury [32] confirming our results even though there were some methodological differences and the mouse models used in the studies were not fully comparable. Dai et al. used ADR at high dose whereas we used a lower dose in C57BL/6J mouse strain,
which may explain the more severe albuminuria described in their study. Furthermore, Dai et al. used a podocin promoter without inducible regulation to create their KO mouse line and thus turned off the expression of β-catenin in podocytes already during embryonic development at the capillary loop stage of nephrogenesis when podocin expression begins. We, however, deleted β-catenin gene in adult mouse podocytes at the age of 8 weeks. It may be that deletion of β-catenin during development generates adaptive processes which may have a different effect on the development of albuminuria than switching off β-catenin transcription in the adulthood.

Besides participating in cadherin-mediated cell adhesion, β-catenin plays a central role in Wnt/wingless signaling pathway. Upon activation, β-catenin translocates to the nucleus and together with T-cell factor/leucocyte-
enhancer factor 1 activates transcription of several genes [27]. In general, Wnt signalling cascades activate various cellular events during kidney development [35]. Texeira et al. [25] and Dai et al. [32] have shown that β-catenin translocates to the nucleus upon ADR treatment indicating activation of Wnt signalling in the ADR model. In this study, we observed β-catenin protein expression in ADR-treated β-catwt/Cre mouse glomeruli, but were unable to detect β-catenin in the nucleus. Furthermore, active dephosphorylated β-catenin or phosphorylated GSK-3β, which are markers for activation of Wnt signalling [36,37] were not up-regulated in β-catwt/Cre mouse kidneys after ADR treatment indicating that Wnt signalling is not activated in our mouse model. We cannot, however, rule out the possibility that the discrepancy between the studies may be due to different responses of the mouse strains (C57BL/6J in this study and Balb/C in [25]) to ADR or different doses of ADR used in this study and in [32]. Taken together, these findings may indicate that a higher degree of podocyte injury is needed for activation of Wnt signalling.

In ADR-treated β-catwt/Cre control mice, β-catenin immunostaining pattern closely resembled that of the SD proteins suggesting that it localizes to the modified podocyte junctions seen after ADR treatment. Electron microscopic examination indicates that these junctions are located more apically within podocyte foot processes, appear narrower than SD and resemble morphologically the junctional complexes present in the differentiating podocytes [38,39]. Interestingly, it has previously been shown that β-catenin is expressed at a higher level in the junc-

**Fig. 6.** β-Catenin expression in podocytes after ADR treatment. (A) β-Catenin showed podocyte-like staining pattern (arrows) in β-catwt/Cre mouse glomeruli 6 days after ADR injection by immunohistochemistry whereas (B) in β-catfl/Cre mice staining in the podocytes remained negative. (C) Control staining without β-catenin antibody was negative. Asterisk (*) indicates urinary space. (D–E) Stainings of serial sections for β-catenin and CD2AP in β-catwt/Cre mouse glomeruli. (F–G) Higher magnifications of the regions indicated in D–E show that β-catenin and a podocyte marker CD2AP were expressed in the same cells. Magnification, ×630. (H) Quantitative immunoblot analysis showed that the level of active β-catenin was similar in β-catwt/Cre and β-catfl/Cre mouse kidneys. Furthermore, phospho-GSK-3β was not expressed in β-catwt/Cre and β-catfl/Cre mouse kidneys after the ADR treatment. E12 mouse embryo lysate was used as a positive control and showed expression of active β-catenin and phospho-GSK-3β. The expression of active β-catenin and phospho-GSK-3β were normalized to α-tubulin expression, and statistical analysis was done by t-test for significance at the P < 0.05 level. Data are presented as mean ± SEM.
tional complexes in developing podocytes [14]. Even though we could not quantify the changes in β-catenin expression in podocytes between ADR-treated β-catfl/fl/Cre and β-catwt/wt/Cre mice, it is tempting to speculate that ADR treatment specifically alters β-catenin expression. Cadherin superfamily member Fat is also expressed at higher levels both in immature podocytes and in injured podocytes in rat puromycin aminonucleoside nephrosis model [40]. Therefore, it was hypothesized that Fat may play a role in remodelling the podocyte junctions both during differentiation and injury. It is tempting to speculate that β-catenin could act in a similar way.

Disruption of the SD after ADR treatment suggests that the expression of key proteins, which are critical for the integrity of the SD could be changed. However, our results showed remarkable stability in the expression levels and localization of podocin, nephrin, ZO-1 and cadherins in both ADR-treated control and β-catenin-deficient mice. This is not surprising since loss of nephrin in man and mouse leading to missing SD structures does not result in changes in the expression of SD proteins including podocin, ZO-1, CD2AP and P-cadherin [20,41–43]. Furthermore, Jeansson et al. also reported that nephrin mRNA expression is not altered after high-dose ADR treatment in C57Bl/6J mouse strain [44].

**Conclusion**

In conclusion, this study shows that lack of β-catenin in mature podocytes protects them against ADR-induced damage and albuminuria. This suggests that β-catenin may be involved in ADR-induced podocyte foot process effacement, disruption of the SD and consequent albuminuria. Additional investigations are needed to elucidate the molecular mechanisms underlying the β-catenin-dependent podocyte injury in ADR nephropathy.

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