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# 令和2年度愛媛大学プロテオサイエンスセンター共同研究報告書

令和3年2月5日

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1. 研究課題

ダイナミンによる細胞骨格制御機構とその破綻に関わる病態解明

2. 研究組織

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3.研究成果

別紙のとおり

#### 研究課題名

ダイナミンによる細胞骨格制御機構とその破綻に関わる病態解明

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#### 研究目的

ほ乳類のダイナミンは、分子量約 100kDa の GTPase であり、細胞膜やアクチン線維上で重合し、自身の GTPase 活性と共役してダイナミックに構造を変化し、細胞膜やアクチン線維を「曲げ」「絞り」「切り」「束ねる」、いわゆるメカノエンザイムとして働くことが知られている。ダイナミンの変異は、シャルコー・マリー・トゥース(CMT)病を含む一部の遺伝性疾患の原因となっている。本共同研究は、腎臓糸球体ポドサイトに発現しているダイナミン1及びダイナミン2の細胞内機能を調べることを目的とした。本年度は、論文投稿後、追加実験に注力した。

## 研究成果

①ダイナミン1は、直接微小管を束ねる。

腎糸球体ポドサイトに発現するダイナミン1が、微小管の配向と安定性に関与するこ とを発見した。今年度は、主に in vitroの系を用いてダイナミン1による微小管の形 態変化を負染色と透過型電子顕微鏡を用いて調べた。in vitroの実験では、ヒトダイ ナミン1、ラットダイナミン2、ラットダイナミン2CMT 変異体をコムギ胚芽無細胞 タンパク発現系にて発現、精製した。



スケール 625 nm (上)、400 nm (下)

図. コムギ胚芽無細胞タンパク発現系を用いて発現、 精製したダイナミン1は微小管に結合し、東化に関わ る。微小管のみ(上)、微小管+ダイナミン1(下) の電顕像。

精製したダイナミン1タンパクと、試験管内 で調製した微小管を混合し、微小管の形態を負 染色と電子顕微鏡で組み合わせて観察した。ダ イナミン1は、微小管上で規則的に重合しダイ ナミン1が結合した微小管同士が束となった。 この結果から、ダイナミン1は、直接微小管を 束ねる能力があることが判明した(左図下)。

②ダイナミン1によって束化した微小管は脱重合速度が減少する。

ダイナミン1によって東化された微小管が安定化するのか否かを、ダイナミン1の存 在または非存在下で、チューブリン脱重合促進剤(カルシウムイオン、 podophyllotoxin)による脱重合キネティクスを計測することで検討した。微小管の分 解速度は、Microtubule depolymerization assay kit (Cytoskeleton 社)を用いて蛍 光プレートリーダーにて測定した。精製したダイナミン1は、2つの脱重合促進剤に よるチューブリンの脱重合を、濃度依存的に抑制した。

昨年までの結果を合わせ、ダイナミン1が、微小管を束ねることで微小管を安定化させ、ポドサイトの形態、機能維持に働いていることが明らかになった。本研究は、米国実験生物学連合会の学会誌である FASEB Journal の 2020 年 12 月号に掲載され、さらに本論文が 12 月号表紙に採択された。

# 成果発表

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題: Dynamin 1 is important for microtubule organization and stabilization in glomerular podocyte

掲載誌: The FASEB Journal, 34, 16449-16463, 2020

# 今後の課題

本研究では、ポドサイトの細胞機能を含め、ダイナミン1及び2の細胞骨格制御機構 の解析を行っている。まずは、ダイナミンによる微小管制御機構の一端を明らかにす ることができた。本研究と並行して高島博士との共同研究であるダイナミン2による アクチン制御機構の解析も進んでいる。ダイナミン2一アクチン線維の構造も明らか にされつつある。今後、議論を進めつつ、さらに研究を遂行する予定である。

# THE **FASEB**JOURNAL

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Dynamin 1 is important for microtubule organization and stabilization in glomerular podocytes. See page 16449.





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#### **Cover Legend**

Radially arranged bundles of microtubules appear in a differentiated mouse podocyte clone (MPC). La et al. demonstrate that dynamin 1 is responsible for bundling and stabilizing microtubules in a differentiated podocyte. Presence of dynamin 1 on the microtubule bundles is revealed by doubleimmunofluorescent staining of a differentiated MPC for dynamin 1 (red) and α-tubulin (green). The cell was pre-treated with Triton X-100 to remove cytosolic proteins.

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#### **RESEARCH ARTICLE**



# Dynamin 1 is important for microtubule organization and stabilization in glomerular podocytes

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#### Abstract

Dynamin 1 is a neuronal endocytic protein that participates in vesicle formation by scission of invaginated membranes. Dynamin 1 is also expressed in the kidney; however, its physiological significance to this organ remains unknown. Here, we show that dynamin 1 is crucial for microtubule organization and stabilization in glomerular podocytes. By immunofluorescence and immunoelectron microscopy, dynamin 1 was concentrated at microtubules at primary processes in rat podocytes. By immunofluorescence of differentiated mouse podocytes (MPCs), dynamin 1 was often colocalized with microtubule bundles, which radially arranged toward periphery of expanded podocyte. In dynamin 1-depleted MPCs by RNAi,  $\alpha$ -tubulin showed a dispersed linear filament-like localization, and microtubule bundles were rarely observed. Furthermore, dynamin 1 depletion resulted in the formation of discontinuous, short acetylated  $\alpha$ -tubulin fragments, and the decrease of microtubule-rich

Abbreviations: Ac-Tu, acetylated tubulin; AP-2, adaptor protein 2; CHC, clathrin heavy chain; Diff, differentiated; Dyn, dynamin; FP, foot process; GBM, glomerular basement membrane; GTP, guanosine triphosphate; MPC, mouse podocyte clones; MT, microtubule; N-SIM, Nikon structured illumination microscopy; PB, phosphate buffer; PBS, phosphate buffered saline; PIPES, Piperazine-1,4-bis(2-ethanesulfonic acid); PP, primary process; siRNA, small interfering RNA; Undiff, undifferentiated; WT1, Wilms tumor 1; α-Tu, alpha tubulin.

The Mon La and Hiromi Tachibana contributed equally to the work.

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protrusions. Dynamins 1 and 2 double-knockout podocytes showed dispersed acetylated  $\alpha$ -tubulin and rare protrusions. In vitro, dynamin 1 polymerized around microtubules and cross-linked them into bundles, and increased their resistance to the disassembly-inducing reagents Ca<sup>2+</sup> and podophyllotoxin. In addition, overexpression and depletion of dynamin 1 in MPCs increased and decreased the nocodazole resistance of microtubules, respectively. These results suggest that dynamin 1 supports the microtubule bundle formation and participates in the stabilization of microtubules.

#### **KEYWORDS**

dynamin, microtubules, podocyte, primary process

# **1** | INTRODUCTION

Glomerular podocytes are highly differentiated epithelial cells that line the urinary side of the glomerular basement membrane and participate in filtration. Podocytes have a complex architecture comprised of major primary processes that branch to form secondary and tertiary foot processes that interdigitate with those of neighboring podocytes to form and maintain the glomerular slit diaphragms.<sup>1</sup> Hence, podocytes are supported by a network of abundant cytoskeleton components, including microtubules, intermediate filaments, and actin filaments. Although the primary foot processes of podocytes are enriched with microtubules,<sup>2</sup> actin filaments are the major components.<sup>3</sup> Proper regulation of these cytoskeletal components is crucial to maintain podocyte morphology and function.

Three isoforms of dynamin exist in mammals.<sup>4</sup> Dynamin 1 is expressed mainly in the brain, whereas dynamin 2 is expressed ubiquitously, and dynamin 3 is localized to the brain, lung and testis.<sup>5</sup> Dynamins 1-3 contain an N-terminal GTPase, a bundle signaling element, a stalk domain, a phosphoinositide-binding pleckstrin homology domain, and a C-terminal proline and arginine-rich domain.<sup>6,7</sup> The latter domain interacts with proteins that contain the Src-homology-3 domain. All dynamins function in endocytosis by participating in membrane fission<sup>8</sup> and are also involved in regulation of the cytoskeleton. Dynamin interacts directly and indirectly with actin to regulate its dynamics<sup>9</sup> in lamellipodia and dorsal membrane ruffles,<sup>10,11</sup> invadopodia,<sup>12</sup> podosomes,<sup>13</sup> growth cones,<sup>14-16</sup> and phagocytic cups.<sup>17,18</sup> Furthermore, dynamin 1 binds directly to microtubules, and this binding stimulates its GTPase activity.<sup>19,20</sup> A Charcot-Marie-Tooth disease-related mutation in dynamin 2 (555 $\Delta$ 3) is implicated in the dynamic instability of microtubules<sup>21,22</sup>; however, the physiological role of dynamin in microtubule regulation remains to be elucidated.

Recently, dynamin has been implicated in maintaining the integrity and structure of the glomerular filtration barrier. Podocyte-specific double knockout of dynamins 1 and 2 in mice results in severe proteinuria and renal failure.<sup>23</sup> In addition, a reduction in cellular dynamin levels via induction of cathepsin L expression causes proteinuria in mice.<sup>24</sup> Dynamin has been implicated in the turnover of nephrin on the surface of podocyte foot process via endocytosis,<sup>23</sup> as well as in maintenance of the structure of foot processes via direct and indirect interactions with actin filaments.<sup>25</sup> Furthermore, enhancement of dynamin oligomerization by Bis-T-23 increases stress fiber and focal adhesion formation in podocytes, resulting in a reduction in the level of proteinuria in several animal models.<sup>26</sup>

Dynamins 1-3 are translated from three separate genes but have similar domains and functions. In podocytes, dynamin 1 is thought to have a similar function to that of dynamin 2. However, a recent study by Khalil and colleagues<sup>27</sup> revealed that the expression patterns of dynamins 1 and 2 differ prior to the onset of proteinuria, suggesting the distinct roles of these isoforms. Consequently, the role of dynamin 1 in podocytes requires further clarification.

In this study, we investigated the function of dynamin 1 in podocytes, and observed that it largely colocalizes with acetylated microtubule bundles in differentiated mouse podocytes (MPCs). Depletion or overexpression of dynamin 1 in differentiated MPCs affected microtubule stabilization. In addition, an in vitro assay revealed tight microtubule bundle formation caused by direct binding of dynamin 1, resulting in enhanced microtubule stabilization. These results provide evidence suggesting that dynamin 1's importance in microtubule regulation.

# 2 | MATERIALS AND METHODS

# 2.1 | Antibodies and reagents

Rabbit anti-Wilms tumor 1 (anti-WT1) antibody (cat#ab89901), rabbit monoclonal anti-dynamin 1 (cat#ab52611) and Alexa Fluor 488-conjugated rat anti-tubulin antibody (cat#ab195883) were purchased from Abcam Plc (Cambridge, UK). The mouse monoclonal clathrin heavy chain antibody (clone X22, cat#MA1-065), mouse monoclonal anti-alpha-adaptin antibodies (cat#MA1-064), rabbit polyclonal antibodies against mouse IgG (cat#31450) and goat IgG (cat#31402), rabbit polyclonal anti-dynamin 1 antibody (cat#PA1-660) and goat polyclonal antibody against rabbit IgG (cat#31460) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The mouse monoclonal antibodies against beta-actin (cat#A5441), alpha tubulin (clone B-5-1-2, cat#T5168), acetylated tubulin (clone 6-11B-1, cat#T6793), mouse anti-acetylated tubulin antibody (cat#T7451), and the Flag tag (cat#F1804) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The goat polyclonal antibody against dynamin 2 (cat#sc-6400) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The mouse monoclonal antibody against synaptopodin (clone G1D4, cat#65194) was purchased from PROGEN Biotechnik GmbH (Heidelberg, Germany). Alexa Fluor 488- (cat#A21206) or Alexa Fluor 555- (cat#A31572) conjugated donkey anti-rabbit IgG, Alexa Fluor 555-(cat#A31570) conjugated donkey anti-mouse IgG, Alexa Fluor 568- (cat#A11057) conjugated donkey anti-goat IgG, Alexa Fluor 488-conjugated goat anti-mouse IgG antibody (cat#A11001), Alexa Fluor 594-conjugated goat anti-rabbit IgG antibody (cat#A11037), and Alexa 488-labeled phalloidin (cat#A12379) were obtained from Thermo Fisher Scientific. Podophyllotoxin (cat#ab142606) was purchased from Abcam Biochemicals. Nocodazole (cat#M1404) was purchased from Sigma-Aldrich.

# 2.2 | Cell culture

The conditionally immortalized mouse podocyte cell line was cultured as described previously.<sup>28</sup> Briefly, the cells were cultured on type I collagen-coated plastic dishes (cat#356450; Corning Inc, NY, USA) in RPMI 1640 medium (cat#189-02025; Fujifilm Wako Pure Chemicals Co. Ltd., Tokyo, Japan) containing 10% of fetal bovine serum (cat#10100147, Thermo Fisher Scientific), 100 U/mL penicillin, 100 µg/mL streptomycin (cat#15140122, Thermo Fisher Scientific), and 50 U/mL mouse recombinant  $\gamma$ -interferon (cat#315-05; PreproTech, Rocky Hill, NJ, USA), and were maintained at 33°C and 5% CO<sub>2</sub>. For differentiation, podocytes were cultured at 37°C in medium lacking  $\gamma$ -interferon for 7-14 days. Under these conditions, the cells stopped proliferating and were positive for synaptopodin.

For primary mouse podocyte cell culture, isolation of podocytes from day 3 in control and dynamin  $1^{-/-}$  dynamin  $2^{-/-}$  Pod-Cre (*pod-Dnm*-DKO) mice were performed as described previously.<sup>23</sup> Briefly, mice glomeruli isolated using the Dynabeads (cat#DB14011, Thermo Fisher Scientific) perfusion was minced with a sterile razor, digested with

collagenase A (5 mg/mL, cat#10103586001, Merck KGaA) containing DNase (0.2 mg/mL, Merck KGaA) for 30 minutes at 37°C under 5% CO2 in cell culture incubator. The digested glomeruli were filtered through a 70-µm cell strainer (cat#352350, Corning Corp.), plated on type I collagen-coated dishes in RPMI 1640 medium (cat#11875-093, Thermo Fisher Scientific) supplemented with 10% FBS, 100 U/mL penicillin/streptomycin, 10 mM HEPES, 1 mM sodium bicarbonate, and 1 mM sodium pyruvate, pH7.4. Subculture of primary podocytes was performed by detaching the glomerular cells with 0.05% trypsin/EDTA (cat#25300054, Thermo Fisher Scientific) at 80%-90% confluency, followed by sieving through a 40-µm cell strainer (cat#352340, Corning Corp.). Primary podocyte enrichment was confirmed by anti-WT1 staining (a specific marker for podocyte), and passage 1 was used in all the experiments.

## **2.3** | **Purification of recombinant proteins**

His-tagged dynamin 1 was expressed using the Bac-to-Bac baculovirus expression system (Thermo Fisher Scientific) and purified as described previously.<sup>16</sup> The purified dynamin solutions were concentrated using Centriplus YM50 (cat#4310; Merck-Millipore, Darmstadt, Germany). Histagged rat dynamin 2 was expressed using a wheat germ cell-free expression system (CellFree Sciences, Matsuyama, Japan). Dynamin 2 was resolved in 100 mM NaCl, 50 mM Tris, 500 mM imidazole, pH8.0, and stored at 4°C until use.

# **2.4** | SiRNA-mediated interference and transfection

The pre-annealed siRNA mixture for mouse dynamin 1 (cat#L043277010010) and the negative control (cat#D0018101005) siRNA were synthesized and purified by Dharmacon Inc (Lafayette, CO, USA). Four siRNAs targeting independent sequences of mouse dynamin 1 were mixed: sense, 5'-GCGUGUACCCUGAGCGUGU-3'; oligo 1 oligo 2 sense, 5'-UGGUAUUGCUCCUGCGACA-3'; oligo 3 sense, 5'-GGGAGGAGGAGGAGGAGCGAAU-3'; oligo 4 sense, 5'-GCUGAGACCGAUCGAGUCA-3'. Scrambled RNA with no significant sequence homology to the mouse, rat or human dynamin 1 gene sequence was used as the negative control. Undifferentiated MPCs were transfected with the siRNAs using Lipofectamine RNAiMax reagent (cat#13778-150, Thermo Fisher Scientific). The cells were seeded into type I collagen-coated 6-well plates (cat#356400, Corning Inc) at a density of  $5 \times 10^4$  cells/ well. One day later, each well was incubated for 6 hours with 60 pmol siRNA and 18 µL RNAiMax in Opti-MEM (cat#31985070, Thermo Fisher Scientific) containing

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 $\gamma$ -interferon. Subsequently, the transfection medium was replaced with fresh medium containing  $\gamma$ -interferon. Following 72 hours, a second transfection was performed, and the cells were cultured for another 72 hours. It was confirmed that all the four siRNAs individually reduced the expression of dynamin 1 (Figure S1). To enable differentiation, the cells were plated into new culture dishes and maintained in medium lacking  $\gamma$ -interferon at 37°C for 7 days.

The expression vector harboring Flag-tagged human dynamin 1 (Gene ID1759) was generated using Gateway cloning technology (Thermo Fisher Scientific). The vector was then transfected into cells using Lipofectamine LTX reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. After transfection for 2 days, the cells were cultured in differentiation medium for a further 7 days.

## 2.5 | Immunohistochemistry

Under sevoflurane anesthesia, 7-week-old male Wister rats (Shimizu Laboratory Supplies Co., Kyoto, Japan) were perfusion-fixed with 4% paraformaldehyde and 20% sucrose in phosphate-buffered saline (PBS; 150 mM NaCl, 10 mM phosphate buffer, pH7.4). The kidney was then cut into slabs and fixed with the same fixative at 4°C for 16 hours. The fixed kidney was cryoprotected with 18% of sucrose and frozen-sectioned at 3  $\mu$ m thickness. Dissected kidney from 2-day-old rats under sevoflurane anesthesia was fixed in the same fixative, and was frozen-sectioned at 3  $\mu$ m thickness. The sections were double stained as described previously.<sup>29</sup>

# 2.6 | Immunoelectron microscopy

For immunoelectron microscopy of glomeruli, 7-weekold male rats were anesthetized and fixed by perfusion of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH7.4)(PB). The kidneys were dissected and processed for embedding in LR White (London Resin Company Ltd., Berkshire, UK). Ultrathin sections (80 nm thickness) were stained with rabbit polyclonal anti-dynamin 1 antibodies (1:10) at 4°C for 16 hours, followed by goat anti-rabbit IgG conjugated with 10 nm gold (1:50, cat#EMGAR10; BBI Solutions, Cardiff, UK) at 4°C for 16 hours. Pre-embedding immunoelectron microscopy of cultured podocyte was performed as described previously.<sup>16</sup> Briefly, differentiated MPCs were fixed with 4% paraformaldehyde in 0.1 M PB, pH7.4 for 15 minutes, and then, washed once. Cells were permeabilized with 0.25% saponin in 0.1 M PB for 30 minutes. After incubation in blocking solution (1% bovine serum albumin and 10% goat serum in 0.1 M PB) for 15 minutes, samples were incubated with rabbit monoclonal anti-dynamin 1 antibody (ab52611, 1:15) diluted in blocking solution at 4°C for 16 hours, washed with 1% bovine serum albumin in 0.1 M PB five times, incubated with 1.4 nm gold conjugated with secondary antibodies (1:50, cat#2002, Nanoprobes Inc, NY, USA), and then, fixed with 1% glutaraldehyde in 0.1 M PB for 10 minutes. The gold particles were developed with silver enhancement kit (cat#2012, Nanoprobes Inc). The samples were postfixed with 0.5% OsO<sub>4</sub> in 0.1 M sodium cacodylate buffer for 90 minutes, dehydrated, and embedded in Epon 812 (cat#341; Nissin EM Co., Ltd., Tokyo, Japan) for ultrathin sectioning. The sections were observed with a Hitachi H-7650 transmission electron microscope (Hitachi High-Tech Corp., Tokyo, Japan).

## 2.7 | Fluorescent microscopy

MPCs were fixed with 4% paraformaldehyde and stained by immunofluorescence as described previously.<sup>16</sup> For Triton X-100 treatment, differentiated MPCs were incubated with 1% Triton X-100 in Brinkley reassembly buffer (BRB80; 80 mM PIPES, 4% polyethylene glycol 8000, 2 mM MgCl<sub>2</sub>, and 0.5 mM EGTA, pH7.0) for 5 minutes at 37°C.<sup>22</sup> The cells were washed once with BRB80 without Triton X-100, and then, followed by immunofluorescence. For nocodazole treatment in differentiated MPCs, cells were incubated with 10 µM nocodazole (cat#M1404, Sigma-Aldrich) at 37°C for 5 or 10 minutes, and then, fixed with 4% paraformaldehyde in PBS. Dynamin 1, Flag-dynamin 1 and α-tubulin were visualized by double-immunofluorescence. Samples were examined using a spinning disc confocal microscope system (X-Light Confocal Imager; CREST OPTICS SPA, Rome, Italy) combined with an inverted microscope (IX-71; Olympus Optical Co., Ltd., Tokyo, Japan) and an iXon+ camera (Oxford Instruments, Oxfordshire, UK). The confocal system was controlled by MetaMorph software (Molecular Devices, Sunnyvale, CA, USA). When necessary, images were processed using Adobe Photoshop CS3 or Illustrator CS3 software. For super-resolution microscopy, N-SIM system was used (NIKON Corp., Tokyo, Japan).

Primary cultured control and *pod-Dnm*-DKO podocytes on type I collagen-coated coverslips were washed with PBS, fixed with 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, blocked with 3% BSA at RT for 1 hour, then, incubated with the appropriate primary antibodies at 4°C for overnight, followed by incubation with Alexa Fluor 488- and/ or Alexa Fluor 594-conjugated secondary antibodies. Images were taken by an Andor CSU-WDi spinning disc confocal microscope equipped with a Nikon Eclipse Ti-E CFI plan apochromat lambda X60 oil immersion objective for immunofluorescence analysis, and images were processed using the NIH image J software (version 1.52t) or Adobe Photoshop CS6.

# **2.8** | Quantification of actin bundles by a low speed sedimentation assay

Non-muscle actin (cat#APHL99, Cytoskeleton Inc, Denver, CO, USA) was polymerized in F-buffer containing 10 mM Tris-HCl, 0.5 mM DTT, 0.2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 50 mM KCl, and 0.5 mM ATP, pH7.5, for 1 hour. Dynamin 1 at 1  $\mu$ M was then incubated with 2  $\mu$ M F-actin in 50 mM KCl or 150 mM KCl containing F-buffer for 1 hour. Actin bundles were sedimented by low-speed centrifugation, at 14 000 *g* for 1 hour. The pellet and supernatant were separated by SDS-PAGE, stained with SYPRO Orange (cat#S6650, Thermo Fisher Scientific), and quantitated by densitometry using Image J. All steps were carried out at room temperature.

# 2.9 | Tubulin disassembly assay

Tubulin disassembly was quantitatively analyzed using a tubulin polymerization assay kit (cat#BK011P; Cytoskeleton Inc). A 36.3  $\mu$ M solution of porcine-brain derived tubulin was prepared in PIPES buffer (80 mM PIPES, 2 mM MgCl<sub>2</sub>, 0.5 mM EGTA, 1 mM GTP, 15% glycerol, pH6.9) containing 5.5  $\mu$ M DAPI. The solution was incubated at 37°C for 1 hour to polymerize tubulin. Subsequently, dynamin 1 was added to the preformed microtubules at the indicated concentrations, and the solution was incubated at 37°C for a further 30 minutes. The fluorescence intensity originating from microtubules (emission: 450 nm; excitation: 350 nm) was monitored with a fluorescent microplate reader (MTP-600F; Corona Electric Co. Ltd., Ibaraki, Japan). The disassembly of tubulin was initiated by adding 2 mM CaCl<sub>2</sub> or 10  $\mu$ M podophyllotoxin.

# 2.10 | Preparation for whole kidney or glomeruli homogenate

Whole kidney of male 6-week-old mice (C57BL/6J) or 7-week-old rat (Shimizu Laboratory Supplies Co., Kyoto, Japan) were separated. Mouse or rat glomeruli were isolated as previously described.<sup>30</sup> The samples were homogenized in PBS containing a protease inhibitor cocktail tablet (cat#11697498001, Roche Diagnostics, Basel, Switzerland) with a Potter-type glass-Teflon homogenizer. The homogenate was centrifuged at 20 000 g for 30 minutes at 4°C. The supernatant was sampled in SDS sample buffer. Samples were boiled for 5 minutes and subjected to Western blotting.

## 2.11 | Electron microscopy

For negative staining, a 8.3  $\mu$ M solution of porcine-brain derived tubulin (cat#BK029, Cytoskeleton Inc) in PIPES buffer was polymerized according to the manufacture's protocol. The paclitaxel stabilized microtubules were incubated with 1  $\mu$ M dynamin 1 at 37°C for 1 hour. Tubulin bundles were formed in vitro as described above. The samples were absorbed to a Formvar- and carbon-coated copper grid and then stained with 3% uranyl acetate in ddH<sub>2</sub>O for 2 minutes. Electron microscopy was carried out using a Hitachi H-7650 transmission electron microscope.

### 2.12 | Morphometry

To assess the colocalization of dynamin 1 with acetylated tubulin, dynamin 2, AP-2, or clathrin, immunostained cells were imaged, and the immunoreactivities within randomly selected areas were measured. Images of control and treated cells stained with an antibody against  $\alpha$ -tubulin were acquired at identical settings with a 40× objective for cells overexpressing dynamin 1 and a 60× objective for dynamin 1-depleted cells. Total pixel intensities per cell were then measured using MetaMorph software. For the quantification of fluorescence, background correction was performed for each image before the measurement.

Outlines of differentiated MPCs or primary cultured mouse podocytes were recognized by cortical actin staining and/or by phase-contrast microscopy observation. Protrusions were defined as processes 4  $\mu$ m or larger in width, and 10  $\mu$ m or larger in length. Protrusions were counted on randomly selected fluorescent images of control and dynamin 1 knock downed MPCs (40 cells), or wild-type (21 cells), and dynamin 1 and 2 double knockout (29 cells) primary mouse podocytes stained for  $\alpha$ -tubulin using Image J.

The fraction of microtubule (MT) bundles to total MTs was determined according to Bai et al.<sup>31</sup> Briefly, we first acquired the sum of fluorescent intensity for  $\alpha$ -tubulin in total cell area from immunofluorescent images using MetaMorph software. Next, the mean fluorescence intensity per pixel for the five-separate single MTs observed in the periphery of each cell was determined. Subsequently, the average value of fluorescence intensity for single MTs per pixel was subtracted from total mean of MTs fluorescence intensity. The resultant sum fluorescent intensity of these putative MT bundles was calculated as a fraction of the total MT fluorescence intensity in using MetaMorph software.



**FIGURE 1** Dynamin 1 is expressed in podocytes. A, Western blotting analyses of dynamin 1 (Dyn1) and dynamin 2 (Dyn2) in rat and mouse kidney homogenates ( $60 \ \mu g \ per \ lane$ ). B, Western blot analyses of dynamin 1 (Dyn1), dynamin 2 (Dyn2), WT1 and  $\beta$ -actin in rat and mouse glomeruli homogenates ( $30 \ \mu g$  for dynamin 1 and dynamin 2, 15  $\mu g$  for WT1, 5  $\mu g$  for  $\beta$ -actin per lane). C, The distribution of dynamin 1 in the renal glomerulus. Sections were co-stained for dynamin 1 (top left) and synaptopodin (top right). Bar: 100  $\mu$ m. D, Immunogold labeling of dynamin 1 in a rat kidney slice. Arrowheads indicate immunogolds for dynamin 1. Bar: 300 nm. PP, primary process; FP, foot process; GBM, glomerular basement membrane

GBM

# 2.13 | Ethics and animal use statement

All experiments and protocols were approved by the institutional animal care and use committee of Okayama University (OKU-2019688, Japan). All efforts were made to minimize animal suffering. After euthanizing mice, whole kidneys were removed.

#### 2.14 | Statistical analysis

Data were analyzed for statistical significance using KaleidaGraph software for Macintosh, version 4.1 (Synergy Software Inc, Essex Junction, VT, USA). Student's *t* tests were used to analyze two groups. P < .05 was considered significant.

# 3 | RESULTS

# **3.1** | Dynamin 1 is present in glomerular podocytes

Western blotting analyses detected expression of dynamin 1, the neuronal isoform, and dynamin 2, which is ubiquitously expressed, in homogenates prepared from rat or mouse kidney (Figure 1A) and glomeruli (Figure 1B). Subsequently, the localization of dynamin 1 in rat kidney was determined by immunohistochemistry. As shown in Figure 1C, dynamin 1 immunoreactivity was clearly observed in glomeruli, at the periphery of glomerular capillaries. Dynamin 1 was also present on the proximal tubules (Figure S2A). Furthermore, dynamin 1 was colocalized with synaptopodin, a podocyte marker, indicating its presence in glomerular podocytes (Figure 1C). Next, we investigated the expression of dynamin 1 and dynamin 2 during glomerulogenesis by immunofluorescence of 2-day-old rat kidney. Both dynamin 1 and dynamin 2 were present in synaptopodin-positive developing glomeruli. The dynamin expressions were weaker at earlier stages, in which synaptopodin is expressed less. While dynamin 2 was expressed in all the cells in the kidney section, dynamin 1 inclined to be expressed in glomeruli (Figure S2B,C). Ultrastructural examination by immunoelectron microscopy of rat kidney revealed that podocyte associated dynamin 1 was mainly localized at the primary processes and the perikarya but not the foot processes (Figure 1D and Figure S3). Immunogold particles were often found in proximity to the filament-like structure such as microtubules, which enriched in primary process (Figure 1D).

# **3.2** | Dynamin 1 accumulates at microtubules in differentiated MPCs

Given the presence of dynamin 1 protein in rat and mouse podocytes, we next examined its expression in a mouse podocyte cell line. MPCs can be differentiated by shifting the culture SEB JOURNAL

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temperature from 33 to 37°C, along with removing  $\gamma$ -interferon from the culture medium.<sup>28</sup> Differentiated MPCs appeared spread-out and extremely large, and the microtubules, which often appeared as loose bundles, extended radially to the cell periphery. Dynamin 1 was present in a punctate pattern on plasma membrane, in cytosol and in nuclei, and partially colocalized with  $\alpha$ -tubulin under the confocal fluorescence microscope (Figure 2A). By super-resolution microscopy, dynamin 1 was visible as dots that were present on both single microtubule and microtubule bundles (Figure 2C). By immunoelectron microscopy, bundles of microtubules radially extending to the cell periphery were evident, and immunogold particles for dynamin 1 were often present on the microtubules (Figure 2D). To further confirm the presence of dynamin 1 on microtubules, we treated cells with Triton X-100 to remove plasma membrane and cytosolic proteins, and the cells were analyzed by immunofluorescence. Under the conditions, dynamin 1 clearly colocalized with microtubules (Figure 2A). The same results were obtained using different anti-dynamin 1 antibody (PA1-660) (Figure 2B). On the contrary, dynamin 2 hardly localized with microtubules (Figure S4). These results suggest that dynamin 1 associates with microtubules.

Next, we examined the effect of dynamin 1 on the intracellular tubulin expression. Western blotting analyses detected dynamin 1 protein in both undifferentiated and differentiated MPCs (Figure 3A). The expression levels of the cytoskeletal proteins β-actin and α-tubulin were similar in undifferentiated and differentiated MPCs (Figure 3A,B), despite the marked difference in size of the cells (Figure 3D). However, the level of acetylated tubulin in differentiated MPCs was approximately ninefold higher than that in undifferentiated cells (Figure 3A,C). Immunofluorescent staining revealed that the radial microtubules were mostly acetylated in differentiated but not undifferentiated MPCs (Figure 3D). Furthermore, dynamin 1 was present as fine puncta and partially colocalized with acetylated tubulin in differentiated MPCs (Figure 3D,E). By contrast, the dynamin 1-positive puncta hardly colocalized with dynamin 2, the clathrin-coated pit marker proteins, AP-2 clathrin heavy chain and actin (Figure S5). We performed low-speed actin cosedimentation assay using dynamin 1 and actin, because dynamin 1 bundles actin filaments, suggesting its role in actin regulation.<sup>32</sup> Dynamin 1 was unable to bundle actin filaments in a physiological ionic strength buffer (Figure S6). These results suggest that dynamin 1 plays a role that is distinct from that of dynamin 2 in endocytosis and actin cytoskeletal regulation.

# **3.3** Dynamin 1 depletion in podocytes causes mislocalization of acetylated tubulin and the decrease of protrusion formation

Although dynamin 1 has been identified as a microtubulebinding protein,<sup>19,20</sup> the physiological significance of this



**FIGURE 2** Dynamin 1 colocalizes with microtubules in differentiated mouse podocyte cell line (MPCs). A, Double immunofluorescent images of dynamin 1 and  $\alpha$ -tubulin using anti-dynamin 1 antibody (ab52611) in differentiated MPCs. Cytosolic and membrane proteins were removed by 1% Triton X-100 (TX100) treatment (bottom panels). Untreated cells were shown (top panels). Bar: 20 µm. B, Double immunofluorescent images of dynamin 1 and  $\alpha$ -tubulin using anti-dynamin 1 antibody (PA1-660) in differentiated MPCs. Cells were treated with (bottom panels) or without (top panels) TX100 as in A. Bar: 20 µm. C, Super-resolution microscopy images of dynamin 1 and  $\alpha$ -tubulin in differentiated MPCs. Bar: 5 µm. D, Immunogold labeling of dynamin 1 (PA1-660) in differentiated MPCs. The boxed areas in the top panel are enlarged (bottom panel). Bar: 1 µm in upper panel, 200 nm in bottom panel

interaction is still unknown. Since dynamin 1 was accumulated on  $\alpha$ -tubulin and acetylated tubulin in differentiated MPCs (Figures 2 and 3), we examined the effect of RNAimediated depletion of dynamin 1 on microtubules. Western blotting analyses revealed that expression of dynamin 1 was selectively knocked down without disturbing the expression of dynamin 2 or  $\beta$ -actin. Depletion of dynamin 1 did not affect the expression of  $\alpha$ -tubulin or acetylated tubulin (Figure 4A). Furthermore, cell size and expression levels of synaptopodin, a marker of podocyte differentiation, were also unaffected by dynamin 1 depletion (Figure S7).

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Next, we determined whether dynamin 1 depletion affects the distribution of  $\alpha$ -tubulin and acetylated tubulin. In control cells, microtubules were loosely bundled and several bundles running radially from the perinuclear region to the cell periphery were evident. The microtubules in dynamin

1-depleted cells were dispersed, and the bundle formation was less prominent, although they were oriented radially as in control cells (Figure 4B). The percentage of microtubule bundles per cell was  $54.9 \pm 1.9$  (n = 21 cells) in the control group and  $39.9 \pm 1.9$  (n = 20 cells) in the dynamin 1-depleted group (Figure 4C). Acetylated tubulin in control cells was present along the radiated microtubules. On the contrary, in the dynamin 1-depleted cells, acetylated tubulin was discontinuous, and therefore, appeared as short fragmented filaments (Figure 4D), suggesting that dynamin 1 might regulate the acetylation state of microtubules. MPCs formed a lot of protrusions enriched with microtubules, and cortical actin was visible at the protrusions. Furthermore, the microtubule-rich protrusions in MPCs were irregularly shaped, not like thin actin bundle-rich filopodia (Figures 4 and S8). Dynamin 1-depleted MPCs rarely formed protrusions as

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FIGURE 3 Increased levels of acetylated tubulin and dynamin 1 colocalization are observed in differentiated MPCs. A, Western blot analyses of dynamin 1 (Dyn1), dynamin 2 (Dyn2), β-actin, α-tubulin (α-Tu) and acetylated tubulin (Ac-Tu) in lysates of undifferentiated and differentiated MPCs (60  $\mu$ g for dynamin 1 and dynamin 2, 5  $\mu$ g for  $\beta$ -actin, 10  $\mu$ g for  $\alpha$ -Tu, and Ac-Tu). B, C, The levels of  $\alpha$ -tubulin (B,  $\alpha$ -Tu) and acetylated tubulin (C, Ac-Tu) in lysates of undifferentiated and differentiated MPCs, as quantified by densitometric analysis. Data are represented as the mean ± SEM (n = 3). \*\*\*\*\*P < .0001. D, Immunofluorescence analyses of dynamin 1 and acetylated tubulin in undifferentiated (top) and differentiated (bottom) MPCs. The boxed areas in the overlay images are enlarged. Bar: 20 µm. E, Quantification of the colocalization of dynamin 1 and acetylated tubulin in differentiated and undifferentiated MPCs. Data are represented as the mean ± SEM of more than 30 cells in three independent experiments. For each sample, colocalization was determined in three randomly selected areas per cell  $(21 \, \mu m^2)$ . \*\*\*\*\*P < .0001

compared to that of control (Figure 4E,F). In addition, podocyte-specific double-knockout of murine dynamins 1 and 2 caused severe proteinuria and renal failure.<sup>23</sup> Therefore, we next examined whether distribution of microtubules and acetylation state of  $\alpha$ -tubulin are altered in primary cultured dynamin double-knockout podocytes. As shown in Figure 4G, the control podocyte had several protrusions containing bundles of microtubules. Mirroring MPC, the microtubules in the bundles were enriched with acetylated tubulin. In dynamin double-knocked out podocytes, the number of protrusion was decreased by approximately 50% as compared to that in control cells (Figure 4H). Overall, these results suggest that dynamin 1 is critical for the regulation of microtubule distributions which is required for protrusion formation.

#### **Dynamin 1 forms stable microtubule** 3.4 bundles in vitro

To investigate the direct effect of dynamin 1 on microtubules, Taxol-stabilized microtubules were incubated in vitro with or without recombinant dynamin 1 at 37°C for 30 minutes, and then, observed by negative staining electron microscopy. In the absence of dynamin 1, the microtubules were dispersed and had a uniform diameter  $(27.3 \pm 0.39 \text{ nm},$ n = 110). However, after incubation with recombinant dynamin 1, the microtubules were often tightly bundled, and dynamin 1-decorated microtubules showed a uniform thin diameter  $(17.2 \pm 0.35 \text{ nm}, \text{n} = 90)$ . Dynamin 1 was periodically arranged on the surface of microtubules, suggesting a helical polymerization around these structures (Figure 5A). These FASEBJOURNAL

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findings suggest that dynamin 1 may regulate microtubule stability via a direct interaction.

Next, we examined the effect of dynamin 1 on tubulin disassembly induced by  $Ca^{2+33}$  or podophyllotoxin<sup>34</sup> in vitro. Tubulin disassembly was monitored by the reduction in fluorescence intensity of diamidino-phenylindole.<sup>35</sup> After incubation of preformed microtubules with dynamin 1,  $Ca^{2+}$  (1 mM) or podophyllotoxin (29  $\mu$ M) was added to the



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**FIGURE 4** Depletion of dynamin 1 causes loss of microtubule bundles and mislocalization of acetylated tubulin. A, Western blotting analyses demonstrates the suppression of dynamin 1 by RNAi in lysates of differentiated MPCs (60 µg for dynamin 1 (Dyn1) and dynamin 2 (Dyn2), 5 µg for  $\beta$ -actin, 15 µg for  $\alpha$ -tubulin ( $\alpha$ -Tu), and 20 µg for acetylated tubulin (Ac-Tu)). B, Immunofluorescence analyses demonstrates a decrease in the amount of loosely bundled microtubules in dynamin 1-depleted MPCs. Dynamin 1 and  $\alpha$ -tubulin were visualized by double-immunofluorescence. Bar: 20 µm. C, The percentage of thick microtubule bundles per cell were determined in 20-21 cells per group (control and dynamin 1-knockdown). Data are represented as the mean  $\pm$  SEM of three independent experiments. \*\*\*\*\**P* < .0001. D, Aberrant distribution of acetylated tubulin in dynamin 1-depleted differentiated MPCs. Dynamin 1 and acetylated tubulin were visualized by double-immunofluorescence. The boxed areas in the overlay images are enlarged. Bar: 20 µm. E, Immunofluorescence analyses demonstrates a decrease in formation of protrusions in dynamin 1-depleted differentiated MPCs. Alpha-tubulin (middle panels) were visualized by double-immunofluorescence. Arrowheads show protrusions. White line shows outline of the cell. Bar: 20 µm. F, The number of protrusions per cell was determined from control or dynamin 1-depleted differentiated MPCs. Data are represented as the mean  $\pm$  SEM. \*\*\**P* < .001. G, Immunofluorescence analyses demonstrates a decrease in formation of protrusions in dynamin 1 and 2 knockout mouse podocyte. Alpha tubulin (top) and acetylated tubulin (bottom) were visualized by double-immunofluorescence. Podocyte marker WT1 (red) were also shown. Bar: 20 µm. H, The number of protrusions per cell was determined from control or dynamins 1 and 2 double knockout podocyte. Data are represented as the mean  $\pm$  SEM.

solution. In the absence of dynamin 1, tubulin was rapidly disassembled after the addition of  $Ca^{2+}$  or podophyllotoxin, and dynamin 1 dose-dependently inhibited the rate of tubulin disassembly (Figure 5B,C). Unlike in the case of dynamin 1, dynamin 2 bound to microtubules irregularly, and it bundled microtubules loosely. Furthermore, dynamin 2 did not change the tubulin depolymerization rate in vitro (Figure S9). These results suggest that dynamin 1 is more potent on microtubule stabilization than dynamin 2.

Finally, to determine the effect of dynamin 1 on stability of microtubules in cells, dynamin 1-overexpressing or dynamin 1-depleted MPCs were treated with nocodazole, a microtubule depolymerizing reagent. Nocodazole-treated cells overexpressing dynamin 1 displayed more bundled microtubules  $(26.0 \pm 3.3\%, n = 31 \text{ cells})$  than nocodazole-treated untransfected cells ( $13.4 \pm 3.7\%$ , n = 28 cells) (Figure 6A). In addition, nocodazole treatment of the untransfected cells partially abolished some of the microtubule arrays. The exogenous dynamin 1 was partially present on the nocodazole-resistant microtubules (Figure 6B). On the contrary, nocodazole-treated dynamin 1-depleted MPCs displayed a smaller number of microtubule bundles ( $12.6 \pm 1.5\%$ , n = 48 cells) than nocodazole-treated control MPCs ( $20.5 \pm 1.6\%$ , n = 45 cells) (Figure 6C). Overall, these results indicate that dynamin 1 stabilizes microtubules in vitro and in vivo.

# 4 | DISCUSSION

Podocytes express two isoforms of dynamin: dynamin 1 and dynamin 2.<sup>23,27</sup> Dynamin 2 in podocytes has been studied mainly in relation to endocytosis and actin regulation<sup>3,23,25,26</sup>; however, the physiological role of dynamin 1 in these cells remains elusive. In the current study, we investigated the intracellular localization and possible roles of dynamin 1 in conditionally immortalized mouse podocytes. We confirmed the expression of dynamin 1 in MPCs as well as renal glomerular podocytes (Figures 1 and 2). In differentiated MPCs, dynamin 1 showed

minimal colocalization with dynamin 2, marker proteins for clathrin-mediated endocytosis, clathrin heavy chain and AP-2, and actin (Figure S5). These results suggested cellular functions that are distinct from those of dynamin 2. Differentiated MPCs formed large spread-out protrusions and contained microtubules that often appeared as loose bundles extending radially to the cell periphery (Figure 2). Differentiated MPCs displayed increased levels of tubulin acetylation and dynamin 1 accumulation at  $\alpha$ -tubulin (Figures 2 and 3).

RNAi-mediated depletion of dynamin 1 resulted in the formation of discontinuous, short  $\alpha$ -tubulin fragments, and defective formation of protrusions. Consistently, dynamins 1 and 2 double-knockout podocytes showed similar microtubule localization and defective formation of acetylated tubulin-rich protrusions (Figure 4), suggesting a role of dynamin 1 in tubulin dynamics. Dynamin 1 bound directly to microtubules and bundled tightly (Figure 5). Furthermore, microtubule bundles containing dynamin 1 were resistant to tubulin disassembly by Ca<sup>2+</sup> or podophyllotoxin (Figure 5). In addition, the presence of dynamin 1 enhanced the resistance of differentiated MPCs to the microtubule destabilizing agent nocodazole (Figure 6). Taken together, these results suggest a direct interaction between dynamin 1 and microtubules, which might be crucial for the regulation of tubulin acetylation and microtubule dynamics in vivo.

Although dynamin 1 has been identified as a microtubule-binding protein in the brain,<sup>19,20</sup> and its role in endocytosis has been well studied,<sup>8</sup> the physiological significance of its microtubule-binding activity has remained unclear. In the current study, we found that dynamin 1 is important for microtubule bundling and stabilization in differentiated podocytes. Similarly, dynamin 2 has been implicated in the dynamic instability of microtubules and microtubule-dependent membrane trafficking in COS cells.<sup>21,22</sup> In both cases, the detailed molecular mechanisms regulating microtubules, including acetylation of tubulin, require further clarification.

Dynamin is also implicated in actin regulation directly or indirectly.<sup>9</sup> Recently, direct actin bundling by dynamin 1 from



FIGURE 5 Dynamin 1 forms tight and thick microtubules, and decreases the rate of tubulin disassembly. A, Electron micrographs of negatively stained microtubules in the presence (top, middle) or absence (bottom) of dynamin 1. Bar: 10 µm (top), 200 nm (middle, bottom). B, C, The kinetics of tubulin disassembly induced by the addition of 1 mM CaCl<sub>2</sub> (B) or 29 µM podophyllotoxin (C) in the presence or absence of dynamin 1 at the indicated concentrations. The rate of tubulin disassembly was measured by the change in fluorescence intensity at 450 nm.

in vitro assay was reported.<sup>32</sup> We confirmed that the dynamin 1 bundles F-actin like as dynamin 2 in low ionic strength buffer, which conditions is widely accepted in the actin fields

(Figure S6). In the study, dynamin 1 hardly colocalized with actin in the cell (Figure S5), and was unable to bundle actin filaments in physiological ionic strength buffer (Figure S6).





(A)



# Nocodazole



**FIGURE 6** Dynamin 1 stabilizes microtubules in differentiated MPCs. A, The sensitivity of dynamin 1-overexpressing differentiated MPCs to nocodazole. After the treatment of nocodazole, microtubules were more evident in MPCs overexpressing dynamin 1 (closed arrowheads) than in untransfected MPCs (open arrowheads). Bar: 20  $\mu$ m. B, Colocalization of over-expressed dynamin 1 on nocodazole-resistant microtubules in differentiated MPCs. Co-immunofluorescence staining as used to visualize  $\alpha$ -tubulin and Flag-dynamin 1. Bar: 20  $\mu$ m. C, The sensitivity of dynamin 1-depleted MPCs to nocodazole. Dynamin 1-depleted MPCs were treated with 10  $\mu$ M nocodazole for 5 minutes. The cells were then fixed, and  $\alpha$ -tubulin was visualized as described in (A). Bar: 20  $\mu$ m

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Thus, there might be some differences between dynamin 1 and dynamin 2 on actin regulation in the cell. Dynamin 2 did not accumulate on microtubules (Figure S4), suggesting that dynamin 1 functions more potently than dynamin 2 in the regulation of microtubules in podocytes.

In protrusions, cortical actin was stained. Dynamin 2 may act on cortical actin regulation required for protrusion formation. The role of dynamin 2 including actin, tubulin and membrane trafficking for protrusion formation needs to be studied in more detail.

In immature rat kidney, dynamin 1 preferentially present in glomeruli with synaptopodin. While dynamin 2 was expressed more evenly throughout kidney. These results suggest the important roles of dynamin 1 on glomerular development (Figure S2B,C). Considering these results, dynamin 1 is likely involved in podocyte morphogenesis including forming primary processes.

Differentiated podocytes have complex architecture with major primary and secondary processes, and a multitude of foot processes that interdigitate with those of neighboring podocytes to form and maintain the glomerular slit diaphragms.<sup>1</sup> Microtubules serve as the main cytoskeleton arrangement in major primary processes and the cell body of podocytes.<sup>36,37</sup> Differentiated podocytes form a number of microtubule bundles that radiate from the perinuclear region to the cell periphery.<sup>36</sup> Several tubulin-binding proteins, such as MAP family proteins, form cross-bridges between microtubules in vitro and in vivo.<sup>38,39</sup> Dynamin 1 can bundle microtubules by forming similar cross-bridges<sup>19</sup> or via dynamin-dynamin interaction in vitro.<sup>40</sup> Tightly bundled microtubules often contain acetylated tubulin, which is characteristically found in stable microtubules.<sup>38,39</sup> In our current study, we found that dynamin 1 accumulated at acetylated tubulin, suggesting a role in the stabilization of microtubules. Therefore, it is possible that dynamin 1 is involved in the formation of major process and supports the morphological structure of podocytes by regulating microtubule dynamics. In addition, microtubule-dependent trafficking of proteins such as nephrin and podocin is crucial for maintaining the structure of secondary foot process.<sup>41</sup> Dynamin 1 could also participate in this trafficking pathway. It was reported recently that the microtubule-binding protein Tau co-organizes microtubule and actin networks.<sup>42,43</sup> Dynamin 1 also could regulate both actin and microtubule dynamics to ensure proper podocyte function. Dynamin 1 and dynamin 2 might act coordinately to maintain the podocyte cytoskeletal structures, which is essential for their filtration function.

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#### **CONFLICT OF INTEREST**

The authors declare no competing interests.

#### AUTHOR CONTRIBUTIONS

H. Yamada, K. Takei, and The Mon La designed the research and wrote the paper; H. Yamada, The Mon La, S. A. Li, S. Seiriki, H. Tachibana, T. Abe, T. Takeda, X. Tian, S. Ishibe, H. Nagaoka, E. Takashima, A. Sakane, T. Sasaki, and S. Makino performed the research; D. Ogawa, K. Asanuma, M. Watanabe, and J. Wada contributed new reagents or analytic tools. All authors read and approved the final manuscript.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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