UPSTATE
MEDICAL UNIVERSITY

Research Article

Title:
New paradigm for cytoskeletal organization in podocytes: Proteolytic fragments of INF2 formin function independently of INF2 actin regulatory activity

Authors:
Mira Krendel[1], David Pruyne[1]

[1] Department of Cell and Developmental Biology, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY 13210, USA

Running title: Active INF2 fragments in podocytes

Word count: 992

Corresponding author: David Pruyne, Department of Cell and Developmental Biology, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY 13210, USA, pruyned@upstate.edu

Key words: actin, formin, FSGS, podocyte, INF2, foot process effacement

Steroid-resistant nephrotic syndrome (SRNS), a condition characterized by the loss of selective glomerular filtration resulting in severe proteinuria, is a common cause of end-stage renal disease, leading to the need for dialysis or kidney transplantation for many patients. The most common histopathological diagnosis in patients with SRNS is Focal Segmental Glomerulosclerosis (FSGS), characterized by the localized sclerosis of some segments of renal glomeruli as well as pathological changes in glomerular visceral epithelial cells (podocytes) and the glomerular basement membrane (GBM). Podocytes have a highly complex cell shape, with numerous projections (foot processes) emanating from the cell body to form attachments to the GBM [1]. The foot processes are interconnected by specialized cell-cell contacts (slit diaphragms), forming an elaborate sieve-like structure that plays an important role in selective glomerular filtration. Foot process formation and the integrity and dynamics of intercellular junctions are regulated by the actin cytoskeleton, which is composed of actin filaments and associated proteins. Thus, it is unsurprising that podocytes depend on the functional actin cytoskeleton, and that mutations in genes encoding regulators of actin organization have been implicated in FSGS [1]. The majority of monogenic cases of FSGS are caused by recessive mutations that result in early onset disease, but many cases of familial FSGS that manifest in early adulthood are inherited in an autosomal-dominant fashion. The most common cause of autosomal-dominant FSGS [2], and the only known genetic cause of Charcot-Marie-Tooth Disease with FSGS [3], are mutations in *INF2*, which encodes a member of the formin family of actin regulatory proteins. In this issue, Subramanian et al. describe a completely novel mechanism of INF2 formin function and provide insight into how mutant INF2 may cause podocyte dysfunction leading to FSGS.

Formins are best known for their ability to enhance the assembly of actin filaments [4]. Formins typically act as dimers whose formin homology FH1 and FH2 domains collaborate to assemble actin monomers into stable nuclei, and to promote the elongation of actin filaments. Among mammalian formins, an N-terminal diaphanous inhibitory domain (DID) typically mediates intramolecular autoinhibition by binding a diaphanous autoregulatory domain (DAD) near the extreme C-terminus. DID/DAD interactions maintain formins in a dormant state, with their actin assembly activity released by binding of a Rho-GTPase to the N-terminus, phosphorylation of the C-terminus, or other mechanisms. Thus, it might be suspected the critical role for INF2 in podocytes is to drive actin assembly, but all known FSGS-causing mutations map to the INF2-DID rather than to actin-organizing domains [5].

So far unique among formins, the DID of INF2 binds strongly to the DAD of formins other than itself, particularly mammalian diaphanous (mDIA) formins, and the INF2-DID inhibits mDIA formins *in trans* [6]. These observations led to the suggestion that the *in vivo* role of INF2 may be to inhibit mDIA-dependent actin assembly. However, one confusing aspect of this model is that the potent actin assembly activity of INF2 is left uninhibited, with the net effect on localized actin assembly unclear. Subramanian and colleagues reveal a possible resolution with discovery that the INF2-DID is liberated from the C-terminal half of the formin by cathepsin-mediated proteolytic cleavage. In cultured podocytes, this allows the N-terminal fragment containing the INF2-DID to accumulate at cell edges together with mDIA formins, while the FH2-containing half remains associated with the endoplasmic reticulum (ER), where it is anchored via prenylation of a C-terminal CAAX-motif. In glomeruli, this spatial segregation is even more pronounced. Using super-resolution microscopy, Subramanian et al. show INF2 N-fragment accumulates in foot processes, while the C-fragment remains in the cell body. While FSGS-associated mutations did not affect proteolytic cleavage of INF2, an INF2 N-fragment bearing the FSGS-associated R218Q mutation had greatly reduced ability to accumulate at cell edges or in foot processes. Importantly, the authors also show the

INF2(R218Q) N-fragment can dimerize with wild-type N-fragment and prevent the wild-type from being recruited to cell edges, providing a potential explanation for the dominant mode of inheritance of INF2-associated FSGS. Finally, pointing to possible significance for INF2 in other kidney diseases, the authors noted that INF2 N-fragment localization correlated with podocyte health in Alport syndrome and in lupus nephritis. That is, among glomeruli from patients with these conditions, INF2 N-fragment was present in normal-appearing foot processes, but absent from regions exhibiting foot process effacement (FPE), while in all cases the C-fragment remained in the podocyte cell body.

Based on these combined results, Subramanian and colleagues propose a model whereby after cathepsin cleavage, the DID-containing INF2 N-fragment enters the foot processes to inhibit resident mDIA formins. Since the FH2-containing C-fragment is retained in the cell body through anchorage to the ER, the net effect is an inhibition of formin-mediated actin assembly in the foot processes. The correlation between INF2 N-fragment mislocalization and occurrence of FPE hints that disinhibition of mDIA formins in absence of INF2-DID might play a role in FPE. Studies from zebrafish provide support for this, where knockdown of INF2 expression results in FSGS-like phenotypes that are reversed by knockdown of fish DIA2 formin [7]. mDIA formins are frequently associated with the assembly of contractile actin filament structures, and cultured podocytes exhibit apparent increased mDIA-dependent contractility with loss of INF2 N-fragment function. Intriguingly, contractile actin structures are normally absent from podocyte foot processes, but have been noted to accumulate at the basal aspect of podocytes in a number of kidney diseases, including FSGS [8]. While it is unclear whether appearance of those contractile structures is cause or effect of FPE, the results presented here hint that in INF2-dependent FSGS, increased contractility may be causative. Important remaining questions are the identity of binding partners for the INF2 N-fragment in glomeruli, and understanding of why the INF2 N-fragment becomes displaced from foot processes in other kidney diseases. These findings also have implications for other cell types, as cathepsin-mediated cleavage of INF2 also occurred in non-podocyte HEK293T cells. Thus, these exciting results indicate that actin cytoskeletal regulation via this novel crosstalk between different formins may be a common mode of formin activity.

Acknowledgments
M.K. is supported by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grant R01DK083345 and D.P. is supported by National Institutes of Health/National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant R01AR064760.

Disclosures
M.K. and D.P. declare no conflicts of interest.

References

1. Scott RP, Quaggin SE: Review series: The cell biology of renal filtration. *J Cell Biol* 209: 199-210, 2015

2. Lepori N, Zand L, Sethi S, Fernandez-Juarez G, Fervenza FC: Clinical and pathological phenotype of genetic causes of focal segmental glomerulosclerosis in adults. *Clin Kidney J* 11: 179-190, 2018

3. Boyer O, Nevo F, Plaisier E, Funalot B, Gribouval O, Benoit G, et al.: INF2 mutations in Charcot-Marie-Tooth disease with glomerulopathy. *N Engl J Med* 365: 2377-2388, 2011

4. Breistprecher D, Goode BL: Formins at a glance. *J Cell Sci* 126: 1-7, 2013.

5. Mademan I, Deconinck T, Dinopoulos A, Voit T, Schara U, Devriendt K, et al.: De novo INF2 mutations expand the genetic spectrum of hereditary neuropathy with glomerulopathy. *Neurology* 81: 1953-1958, 2013

6. Sun H, Schlondorff JS, Brown EJ, Higgs HN, Pollak MR: Rho activation of mDia formins is modulated by an interaction with inverted formin 2 (INF2). *Proc Natl Acad Sci U S A* 108: 2933-2938, 2011

7. Sun H, Al-Romaih KI, MacRae CA, Pollak MR: Human kidney disease-causing INF2 mutations perturb Rho/Dia signaling in the glomerulus. *EBioMedicine* 1: 107-115, 2014

8. Suleiman HY, Roth R, Jain S, Heuser JE, Shaw AS, Miner JH: Injury-induced actin cytoskeleton reorganization in podocytes revealed by super-resolution microscopy. *JCI Insight* 2: e94137, 2017