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GLOMERULAR DISEASE

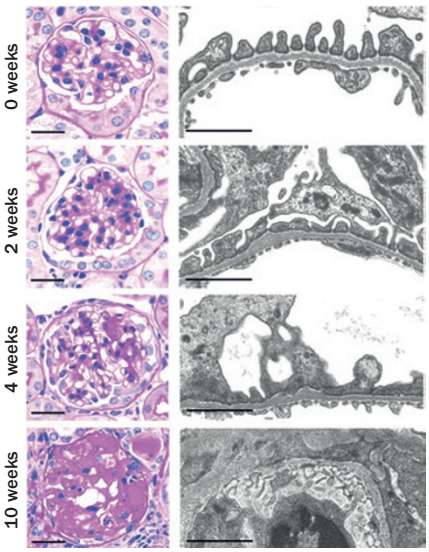
MicroRNA-193a—a new pathogenic player and target in focal segmental glomerulosclerosis

Dysregulation of a single microRNA, miR-193a, induces a molecular cascade that leads to glomerular damage and focal segmental glomerulosclerosis (FSGS) in mice and humans, according to new research published in *Nature Medicine*. "These findings are novel because until now there has been no mechanistic insight into the pathogenesis of the majority of cases of FSGS," says researcher Dontscho Kerjaschki. "Genetic defects in certain constituent proteins, the circulating nephritogenic factor suPAR, and other known factors, together account for less than 50% of FSGS cases."

FSGS is a severe glomerular disease caused by the dysregulation of podocyte proteins and is characterized by the destabilization of podocyte foot processes and glomerular filtration barrier. Although causative factors such as mutations in key podocyte genes have been identified, the pathogenic cause of the majority of cases of FSGS is unknown.

Kerjaschki and colleagues initiated the current project when they found that the induction of miR-193a in transgenic mice resulted in a renal phenotype, characterized by contracted kidneys and the development of albuminuria. "The current work started from a serendipitous observation," Kerjaschki explains. "Our oncology colleagues developed a transgenic mouse in which miR-193a overexpression could be induced by doxycycline, in the hope that it would induce mammary tumours. Instead, the mice developed an accelerated form of FSGS and died within 10 weeks due to renal failure. We were thrilled because this was arguably the first faithful mouse model of this devastating human disease; with such a reproducible model to hand, unravelling the molecular pathogenesis was rather straightforward."

Histological examination of kidney sections from transgenic mice revealed changes in the glomerular architecture 2 weeks after doxycycline exposure. Focal sclerosis developed within 4 weeks and after 10 weeks, glomeruli were globally sclerotic with broad capsular adhesions. Electron microscopy revealed rarification of the slit diaphragms within 2 weeks, with flattening of podocytes and fusion of slit diaphragms by 4 weeks.



Glomerular lesions and podocyte flattening develop progressively 2, 4, and 10 weeks after the induction of miR-193 overexpression, as visualized by Periodic acid–Schiff staining (left) and electron microscopy (right). Permission obtained from Nature Publishing Group © Gebeshuber, C. A. *et al*. *Nat. Med*. doi:10.1038/nm.3142.

To determine the downstream targets of miR-193a, the researchers analysed the expression of glomerular genes following the induction of miR-193a. They identified several genes that had previously been linked to podocytes, including *WT1*, a gene also associated with FSGS. "We next showed that miR-193a targeted a single binding site in the coding region of *WT1*, a master controller of the podocyte phenotype," says Kerjaschki. Co-transfection of miR-193a into HEK293 cells that had been transiently transfected with either wild-type *Wt1* or a mutant *Wt1* with an altered miR-193a binding site, resulted in decreased expression of wild-type, but not mutant *Wt1*.

The researchers next examined the downstream effects of *WT1* suppression on podocytes. They first assessed the ability of miR-193a to induce changes in the levels of 15 genes expressed by podocytes. Among the genes that were downregulated by miR-193a were known targets of *WT1* (*Podx1*, *Nphs1*, *Notch1*, *Vegfa*) and components of the slit diaphragm (*Nphs2*, *Cd2ap* and *Inf2*). Immunofluorescence studies confirmed the loss of *Wt1*, podocalyxin (encoded by *Podx1*) and nephrin (encoded by *Nphs1*) in the glomeruli of transgenic miR-193a mice. Inducible, podocyte-specific deletion of *Wt1* in adult mice also resulted in loss of podocalyxin and nephrin, confirming these podocyte-relevant proteins to be downstream targets of Wt1. Injection of a microRNA-inhibiting locked nucleic acid designed specifically to inhibit miR-193a, increased expression levels of *Wt1*, *Podx1* and *Nphs1*, and lowered albuminuria in transgenic mice overexpressing miR-193.

Kerjaschki and colleagues also quantified miR-193a in isolated glomeruli from 90 individuals with FSGS or other primary glomerular diseases. Expression of miR-193a was significantly higher in the group with FSGS than in other groups. Notably, overexpression of miR-193a was not observed in individuals with hereditary FSGS.

The researchers say that the next important question is to identify the cause of the increased expression of miR-193a in FSGS-affected podocytes. "These studies could open the door to the design of a specifically targeted therapy for FSGS," concludes Kerjaschki.

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Original article Gebeshuber, C. A. *et al*. Focal segmental glomerulosclerosis is induced by microRNA-193a and its downregulation of WT1. *Nat. Med*. doi:10.1038/nm.3142