# Circular RNAs in kidney disease and cancer

*Anton Jan van Zonneveld[1], Malte Kölling[2], Roel Bijkerk[1] and Johan M. Lorenzen[2]* ✉

Abstract | Circular RNAs (circRNAs) are a class of endogenously expressed regulatory RNAs with a single-stranded circular structure. They are generated by back splicing and their expression can be tightly regulated by RNA binding proteins. Cytoplasmic circRNAs can function as molecular sponges that inhibit microRNA–target interactions and protein function or as templates for the efficient generation of peptides via rolling circle amplification. They can also act as molecular scaffolds that enhance the reaction kinetics of enzyme–substrate interactions. In the nucleus, circRNAs might facilitate chromatin modifications and promote gene expression. CircRNAs are resistant to degradation and can be packaged in extracellular vesicles and transported in the circulation. Initial studies suggest that circRNAs have roles in kidney disease and associated cardiovascular complications. They have been implicated in hypertensive nephropathy, diabetic kidney disease, glomerular disease, acute kidney injury and kidney allograft rejection, as well as in microvascular and macrovascular complications of chronic kidney disease, including atherosclerotic vascular disease. In addition, several circRNAs have been reported to have oncogenic or tumour suppressor roles or to regulate drug resistance in kidney cancer. The available data suggest that circRNAs could be promising diagnostic and/or prognostic biomarkers and potential therapeutic targets for kidney disease, cardiovascular disease and kidney cancer.

Intergenic
Region between two protein-coding genes.

Intragenic
Region within a gene.

Intronic
Intron region of a protein-coding gene.

Alu elements
Short stretches of DNA that contain an abundance of transposable elements.

[1]*Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands.*

[2]*Division of Nephrology, University Hospital Zürich, Zürich, Switzerland.*

✉*e-mail: j.m.lorenzen@gmail.com*

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Circular RNAs (circRNAs) are gene-regulatory RNA transcripts with covalently closed circular structures that confer high stability. They are expressed in a cell-specific and organ-specific manner and have critical functions in biology. CircRNAs regulate gene expression by binding to microRNAs (miRNAs) and proteins[1–4]. They were initially described in 1976 as viroids with pathogenic activity towards certain higher plants[5]. The basic concepts of alternative splicing were identified 1 year later, in 1977 (REF.[6]), but were not elucidated as an essential step in circRNA biogenesis until the 1990s[7].

CircRNAs were first described in humans as scrambled exons in 1991 (REF.[7]). For many decades they were considered to be either pathogenic material or the result of low levels of mis-splicing events[5,8–10]. However, in 2012 and 2013, thousands of ubiquitously expressed and covalently closed circRNAs were systematically detected in eukaryotic cells using advanced next-generation sequencing techniques and circRNA-specific bioinformatic algorithms[1–4]. Further studies confirmed abundant, tissue-specific circRNA expression patterns that are well-conserved between mouse and human[2].

In this Review, we provide a comprehensive overview of current basic concepts of circRNA biogenesis and function and highlight current knowledge of their functional roles in kidney disease, cardiovascular complications and kidney cancer. We also outline the therapeutic and diagnostic potential of circRNAs as targets in these diseases.

## CircRNA biogenesis

Most circRNAs (84%) originate from protein-coding genes; 85% align sense and 10% align antisense to exons of known coding and non-coding genes, whereas the remaining 5% align to untranslated regions, introns or unannotated gene loci[3]. CircRNAs are generated by a special form of alternative splicing, termed back splicing (FIG. 1). Precursor-mRNAs are back spliced by joining the 3′ splice sites of downstream exons to the 5′ splice sites of upstream exons[1]. All exons except the first and last can undergo backsplicing[1–3]. The majority of circRNAs derive from mid-positioned exons and contain two to three exons; however, circRNAs deriving from intergenic, intragenic and intronic regions and antisense sequences have been reported[11].

Specific genomic features are required to initiate circRNA formation. First, circRNA exons and their flanking introns have to be exceptionally long, on average three-fold longer than canonical linear RNAs[2]. Second, these long introns must contain inverted complementary sequence elements, such as inverted repeat Alu elements, to bring downstream 5′-donor and upstream 3′-acceptor

**Key points**

- CircRNAs are regulatory RNA molecules with a closed circular structure that are generated by back splicing of precursor mRNAs.
- Functions of cytoplasmic circRNAs include sponging of microRNAs and proteins, scaffolding of enzyme–substrate interactions and acting as templates for protein translation.
- Nuclear-enriched circRNAs can also act as molecular sponges and promote gene expression by interacting with chromatin remodelling complexes and increasing RNA polymerase II activity.
- CircRNAs have been implicated in the pathogenesis of kidney diseases, cardiovascular complications of chronic kidney disease and kidney cancer, and are promising potential therapeutic targets.
- CircRNAs are promising biomarkers of disease owing to their high stability and packaging in extracellular vesicles.
- Potential circRNA-based therapeutic approaches include modulation of native circRNAs and the application of artificial circRNAs with designer molecular functions.

splice sites into close proximity[11,12]. Most circRNAs are located within protein coding genes and contain complete exons, indicating that transcription is mediated by RNA polymerase II[12].

### *Fine-tuning by RNA binding proteins*

RNA binding proteins (RBPs) can fine tune the extent of circRNA formation. For example, muscle blind-like protein 1 (MBNL1) promotes the generation of its own circRNA, circMbl, by direct binding of flanking introns and exons. Increased circMbl expression represses linear Mbl mRNA expression via an autoregulatory mechanism[12]. Another RBP, quaking (QKI), is an alternative splicing factor that promotes circRNA formation. QKI has been shown to regulate the expression of hundreds of circRNAs during transforming growth factor β (TGFβ)-induced epithelial to mesenchymal transition[13].

### *CircRNA expression*

Most circRNAs are expressed at low levels with an abundance that is approximately 5–10% of that of the corresponding linear RNA[14]. However, some circRNAs accumulate in cells owing to their high stability and are able to exceed the expression levels of linear RNAs by 10-fold[1,2]. Highly expressed circRNAs are cell and gene-specific and their levels are not linked to those of the corresponding linear RNA[14].

CircRNAs are predominantly enriched in the cytoplasm[1–3]. Their nuclear export is mediated by the transcription–export complex in conjunction with the exon junction complex[15] and is dependent on their length. Long circRNAs (~1,300 nucleotides) bind to spliceosome RNA helicase DDX39B, whereas short circRNAs (~400 nucleotides) bind to ATP-dependent RNA helicase DDX39A[16]. Both complexes are then recruited by the NTF2-related export protein 1 (NXT1)–nuclear RNA export factor 1 (NXF1) heterodimeric export receptor and released into the cytoplasm through the nuclear pore complex (FIG. 1).

### *CircRNA degradation and exosome release*

CircRNAs are fairly resistant to degradation owing to their circular structure and knowledge of their degradation mechanisms is incomplete. Some circRNAs degrade upon miRNA binding and argonaute-2 (AGO2)-mediated cleavage (FIG. 2a)[4,17], whereas others are degraded by specific RNases following modification with $N^6$-methyladenosine ($m^6A$)[18]. The N6-adenosine-methyltransferase subunit METTL3–N6-adenosine-methyltransferase non-catalytic subunit (METTL14) core complex can modify circRNA transcripts with $m^6A$ in the nucleus, which enables endoribonucleolytic cleavage by YTHDF2–HRSP12–RNase P/MRP in the cytoplasm (FIG. 2b)[19].

Additional decay mechanisms are associated with the secondary structure of circRNAs[20,21]. For example, in the absence of viral infection, ribonuclease L (RNase L) is inactive and RNA duplexes (16–26 base pairs) of circRNAs bind and thereby silence double-stranded RNA-activated protein kinase (PKR). Upon viral infection, RNase L is induced and degrades circRNAs, thereby releasing and activating PKR, which has a role in the early responses of innate immunity. PKR activation is thus a result of circRNA degradation (FIG. 2c).

Another circRNA decay mechanism is associated with the RBPs regulator of nonsense transcripts 1 (UPF1) and Ras GTPase-activating protein-binding protein 1 (G3BP1). These RBPs bind and unwind circRNAs and the helicase activity of UPF1 leads to circRNA degradation[21] (FIG. 2d). This degradation is structure related and not dependent on RNA sequence.

Some circRNAs are packaged into small extracellular vesicles (40–100 nm) and released into the extracellular space upon fusion of multivesicular endosomes with the cell membrane[22–25]. Whether circRNAs can be released in other forms of extracellular vesicles, such as microvesicles and microparticles, remains to be investigated[26]. The levels of circRNAs in exosomes are higher than their corresponding levels in releasing cells[22] (FIG. 2e). Exosomes have been detected in the kidney and are released along the whole nephron[27], suggesting that altered levels of circRNAs packaged into exosomes and detected in urine might deliver direct information about the constitution of the whole kidney. Consequently, urinary circRNAs might be promising non-invasive biomarkers of kidney disease.

## CircRNA functions

High cross-species conservation and the remarkable stability of circRNAs suggest that these RNAs have essential functions. Various cytoplasmic circRNAs have been reported to function as miRNA and protein sponges, protein scaffolds and templates for protein translation. In addition, some nuclear-enriched circRNAs might promote gene expression.

### *Cytoplasmic circRNAs*

***MicroRNA sponging.*** To date, the most extensively investigated function of circRNAs is miRNA sponging (FIG. 3a). CircRNAs can bind to complementary sequences within miRNAs and therefore impede miRNA-mediated repression of target mRNAs. This sponging mechanism enables the target mRNAs to be re-bound by ribosomes and subsequently translated into protein[28]. The elucidation of circRNA-mediated miRNA sponging activities was mainly driven by initial

ground-breaking discoveries regarding the ciRs-7 (also known as CDR1as)–miRNA-7 interaction[3,4]. ciRs-7 is highly expressed in neurons and contains 63 conserved miRNA-7 binding sites[29]. ciRs-7-mediated sponging prevents degradation of miR-7, which is transported to neuronal cell extensions and synapses, where it represses its targets[29,30].

***Protein sponging.*** Crosslinking experiments and subsequent bioinformatic analyses have identified multiple RBP sites in circRNAs, suggesting circRNA–protein interactions, including molecular sponging of proteins[31] (FIG. 3b). Some of these interactions have been experimentally validated. For example, circPABPN1 has been shown to regulate cell proliferation via Hu-antigen R (HUR) sponging[32] and circANRIL has been shown to protect against atherosclerosis by sponging pescadillo homologue 1 (PES1) (discussed further below)[33].

***Protein scaffolding.*** As circRNAs are capable of binding proteins, they can act as molecular scaffolds to enhance the reaction kinetics of enzyme–substrate interactions (FIG. 3c). For example, circFoxo3 is highly expressed in mouse non-cancer cell lines and scaffolds cyclin-dependent kinase inhibitor 1 A (also known as p21) and

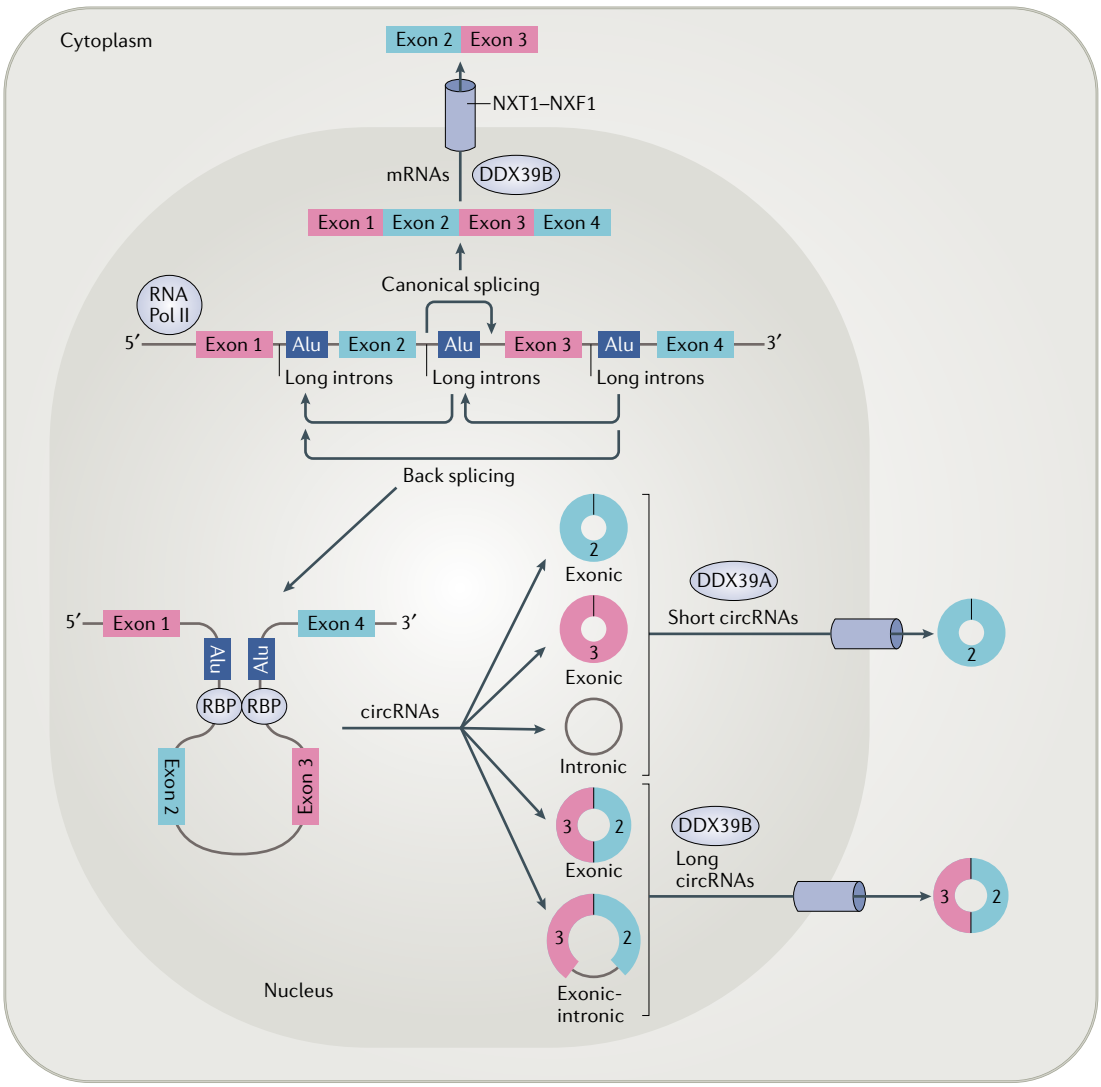


Fig. 1 | **CircRNA biogenesis.** Messenger RNA (mRNA) synthesis occurs via canonical splicing, in which exons are aligned to generate the mRNA. Circular RNAs (circRNAs) are transcribed by RNA polymerase II and generated by back splicing of precursor mRNAs. In this process, 3′ splice sites of downstream exons are joined to 5′ splice sites of upstream exons; all exons except the first and the last can be included. Long flanking complementary introns containing inverted repeat elements, such as Alu repeats, are required for back splicing. Most circRNAs derive from exons; however, circRNAs derived from intergenic, intragenic or intronic regions and from antisense sequences have been reported. CircRNA biogenesis is fine-tuned by *trans*-acting RNA binding proteins (RBPs). Long circRNAs (~1,300 nt) bind to spliceosome RNA helicase DDX39B, whereas short circRNAs (~400 nt) bind to ATP-dependent RNA helicase DDX39A. These complexes are recruited by the NTF2-related export protein 1 (NXT1)–nuclear RNA export factor 1 (NXF1) heterodimeric export receptor and released into the cytoplasm through the nuclear pore complex.

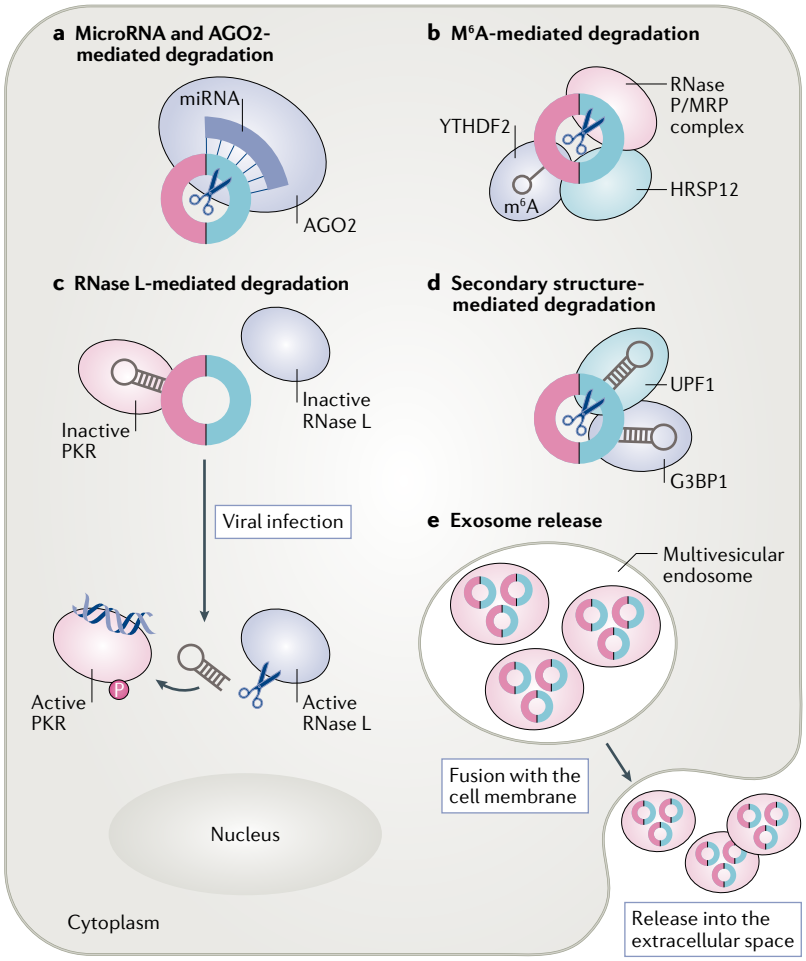


Fig. 2 | **CircRNA degradation and exosome release. a** | Some circular RNAs (circRNAs) can be degraded by specific microRNA (miRNA) binding and subsequent argonaute-2 (AGO2)-mediated RNA cleavage. **b** | CircRNAs that are modified with $N^6$-methyladenosine ($m^6A$) can be recognized and cleaved by the YTHDF2–HRSP12–RNase P/MRP complex. **c** | RNA duplexes (16–26 base pairs) of circRNAs can bind and inhibit the activity of double-stranded RNA-activated protein kinase (PKR). Upon viral infection, RNase L is induced and degrades the circRNAs, thereby releasing and activating PKR, which has a role in the early innate immune response. **d** | Secondary structure-mediated binding of the RNA binding proteins regulator of nonsense transcripts 1 (UPF1) and Ras GTPase-activating protein-binding protein 1 (G3BP1) can unwind circRNAs, enabling their cleavage by the helicase activity of UPF1. **e** | CircRNAs can be packaged into exosomes and released into the extracellular space upon multivesicular endosome fusion with the cell membrane.

cyclin-dependent kinase 2 (CDK2), leading to arrest of cell-cycle progression and cell proliferation[34].

***Protein coding.*** CircRNAs were initially categorized as non-coding RNAs as they seem to lack 5′ caps, poly(A) tails and easily identifiable open reading frames. However, further studies demonstrated possible translation of circRNAs[35]. Translation might occur through rolling circle amplification of circRNAs containing internal ribosome entry sites[14] or of circRNAs that have undergone $m^6A$ modification of their 5′ untranslated regions[15] (FIG. 3d). Rolling circle amplification is a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of DNA or RNA[13]. CircRNAs might therefore function as templates for protein translation.

ircMbl not only has a role in RBP interactions, but also is translated into protein upon starvation and forkhead box protein O (FOXO) activation[35]. circZNF609 is translated by utilizing the start codon of linear zinc finger protein 609 (ZNF609) mRNA and a stop codon generated upon circularization. circZNF609 controls cell proliferation and was upregulated in differentiated myoblasts from patients with Duchenne muscular dystrophy[36]. Furthermore, several circRNAs, including circFBXW7, circSHPRH and circPINTexon2, have been shown to translate tumour suppressor proteins[37–39]. Although thousands of circRNAs containing internal ribosome entry sites and downstream open reading frames have been predicted by computational algorithms[40], translation has been reported for only a few circRNAs. The overall relevance of circRNA translation requires further investigation.

### *Nuclear-enriched circRNAs*

CircRNA sponging activity has also been identified in the nuclear compartment. For example, cia-cGAS acts as a molecular sponge of cyclic GMP–AMP synthase (cGAS) (FIG. 3e) and thereby protects haematopoietic stem cells from cellular exhaustion[41]. Nuclear circRNAs can also promote gene expression. For example, the circRNA FECR1, which is highly expressed in breast cancer cells, can promote gene expression by binding to promoter chromatin complexes and influencing DNA hypomethylation in the CpG islands of the promoter[42] (FIG. 3f). In addition, the circRNAs ci-ankrd52, sircEIF3J and circPAIP2 can increase RNA polymerase II activity, for example, by associating with elongation polymerase II machinery, and therefore promote gene expression[43,44] (FIG. 3g).

## CircRNAs in kidney disease

The role of circRNAs in kidney disease is an area of intensive investigation (TABLE 1). The available data suggest that circRNAs are potential biomarkers of disease activity and therapeutic targets.

### *Hypertensive nephropathy*

In mice, 124 circRNAs were found to be differentially expressed between normal kidneys and kidneys that had been injured as a result of salt-sensitive hypertension[45]. One of the most highly suppressed circRNAs was circNr1h4, which is derived from the *Nr1h4* gene, which encodes the bile acid receptor. The researchers postulated that circNr1h4 sponges miR-155-5p, which acts as a downstream regulator of fatty acyl-CoA reductase 1 (Far1) and showed that sponging of miR-155-5p results in reduced production of reactive oxygen species. Their hypothesis was supported by gain-of-function and loss-of-function studies. These findings suggest that circRNAs might interfere with the cellular stress response in hypertensive nephropathy by influencing pro-inflammatory signalling. Future studies are required to investigate whether targeting of circNr1h4 might be beneficial for patients with hypertensive nephropathy.

Another study reported distinct profiles of dysregulated circRNAs in the kidneys of normotensive and

Rolling circle amplification
An isothermal enzymatic process in which a short DNA or RNA primer is amplified to form a long single-stranded DNA or RNA using a circular DNA template and special DNA or RNA polymerases.

Pyroptosis
A highly inflammatory form of lytic programmed cell death that occurs most frequently upon infection with intracellular pathogens and is likely to form part of the antimicrobial response.

hypertensive rats[46]. A comparison of hypertensive Dahl salt-sensitive and normotensive Dahl salt-resistant rats showed that 318 circRNAs were differentially regulated, whereas 110 circRNAs were differentially expressed in spontaneously hypertensive rats compared with normotensive Wistar Kyoto rats. A number of circRNAs, including, rno_circRNA_014746, rno_circRNA_004804 and rno_circRNA_004811, were validated and confirmed to be dysregulated in the hypertensive rats using quantitative PCR. Whether any of the dysregulated circRNAs are involved in disease-specific signalling remains to be elucidated.

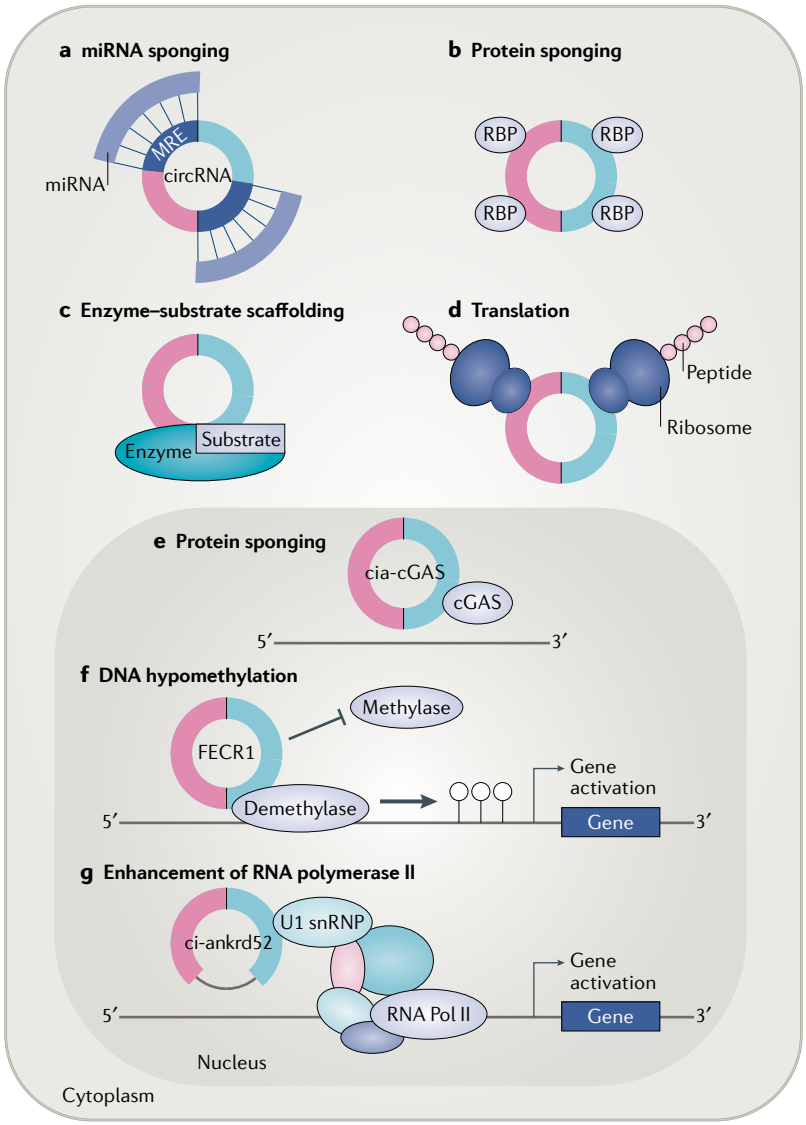


Fig. 3 | **CircRNA functions. a** | In the cytoplasmic compartment, circular RNAs (circRNAs) containing microRNA response elements (MREs) can function as microRNA (miRNA) sponges by binding miRNAs and thereby preventing miRNA-mediated repression of target messenger RNAs. **b** | Specific interactions between circRNAs and RNA binding proteins (RBPs) can sponge the molecular function of the protein. **c** | CircRNAs can act as scaffolds for enzyme–substrate interactions and thereby enhance the reaction kinetics. **d** | CircRNAs with internal ribosome entry sites or $m^6A$ RNA modification of 5′ untranslated regions can be translated via rolling circle amplification. **e** | In the nucleus, cia-cGAS has been reported to sponge cyclic GMP–AMP synthase (cGAS)[41]. **f** | FECR1 can promote gene expression by binding to the gene promoterchromatin complex, thereby inducing demethylases and silencing methylases[42]. **g** | CircRNAs such as ci-ankrd52, sircEIF3J and circPAIP2 can promote gene expression by enhancing RNA polymerase II (RNA Pol II) activity via interaction with small nuclear ribonucleoprotein U1 (U1 snRNP) or promotion of the RNA Pol II elongation machinery.

### *Diabetic kidney disease*

The role of circRNAs in the pathogenesis of diabetic kidney disease has also been investigated. A global expression analysis found that a variety of circRNAs are dysregulated in kidneys of diabetic db/db mice compared with non-diabetic controls[47]. In particular, expression of circRNA_15698 was increased in db/db kidneys and in cultured mesangial cells under high glucose conditions. Expression of this circRNA was enriched in the cytoplasm. Silencing of circRNA_15698 in mesangial cells resulted in reduced expression of collagen I, collagen IV and fibronectin. This circRNA was proposed to function as a sponge for miR-185, which in turn was postulated to regulate TGFβ signalling and extracellular matrix production. This study is the first to demonstrate that a circRNA might interfere with fibrotic remodelling through interaction with its target miRNA. Further studies on the effects of modulation (overexpression and silencing) of circRNA_15698 in mouse models are required to decipher the role of this circRNA in fibrogenesis.

A global expression analysis showed upregulation of circACTR2 in human proximal tubular epithelial cells (HK-2) cells that were exposed to high glucose conditions[48]. Silencing of circACTR2 resulted in reductions in pyroptosis, IL-1β secretion and the expression of collagen IV and fibronectin, which have roles in fibrosis. CircACTR2 is therefore a potential therapeutic target to interfere with regulated cell death and subsequent fibrotic remodelling in diabetic kidney disease.

### *Glomerular disease*

CircRNAs have also been investigated in a number of glomerular diseases. Lupus nephritis is a frequent complication of systemic lupus erythematosus (SLE). Although circRNA expression in patients with lupus nephritis was not investigated, a study that used next-generation sequencing reported that circ_0000479 was the most highly upregulated circRNA in PBMCs from patients with SLE compared with those from healthy individuals[49]. In a validation cohort, circ_0000479 accurately distinguished patients with SLE from healthy people and patients with rheumatoid arthritis. A bioinformatic analysis suggested that circ_0000479 promotes SLE progression by regulating metabolic and Wnt signalling pathways. Moreover, a gene ontology analysis showed that circRNA target genes were involved in biological processes such as RNA splicing, regulation of protein secretion, ATP synthesis-coupled proton transport and the metabolic process. In the cellular component category, enriched terms included intracellular, lysosomal membrane and cytoplasmic exosome. In the molecular function category, enriched terms included protein binding, transferase activity, and nucleic acid binding. circ_0000479 might therefore be a specific biomarker of SLE as well as a downstream effector of important disease-specific signalling pathways that have an impact on metabolism, including the Wnt pathway.

Table 1 | **Dysregulated circRNAs in kidney disease**

| CircRNA | Function | Effect or application | Expression | Ref. |
|---|---|---|---|---|
| ***Hypertensive nephropathy*** | | | | |
| circNr1h4 | miR-155-5p sponge | Increased Far1 expression and reduced production of ROS | Downregulated in the kidneys of mice with salt-sensitive hypertension and in cultured mouse kidney collecting duct cells | 45 |
| rno_circRNA_014746 | Unknown | Potential roles in blood pressure regulation | Upregulated in the kidneys of hypertensive rats | 46 |
| rno_circRNA_004804 | | | | |
| rno_circRNA_004811 | | | | |
| ***Diabetic kidney disease*** | | | | |
| circRNA_15698 | miR-185 sponge | Silencing reduced expression of collagen I, collagen IV and fibronectin | Upregulated in db/db mouse kidneys and in cultured mesangial cells under high glucose conditions | 47 |
| circACTR2 | Unknown | Silencing reduced pyroptosis, IL-1β secretion and fibrosis development | Upregulated in human proximal tubule cells under high glucose conditions | 48 |
| ***SLE and lupus nephritis*** | | | | |
| circ_0000479 | Unknown | Promotes disease activity in SLE by regulating metabolism and Wnt signalling | Upregulated in PBMCs from patients with SLE | 49 |
| circHLA-C | Predicted to sponge miR-150 | Positively associated with proteinuria, kidney function, disease activity scores and glomerular injury | Upregulated in kidney biopsy samples from patients with lupus nephritis | 50 |
| ***Membranous nephropathy*** | | | | |
| circ_101319 | Unknown | Associated with disease activity | Upregulated in PBMCs from patients with membranous nephropathy | 51 |
| ***AKI*** | | | | |
| circ-0114427 | miR-494 sponge | Increased ATF3 expression and IL-6 secretion | Upregulated in mice with cisplatin-induced AKI and in cultured proximal tubular cells exposed to cisplatin | 52 |
| circYAP1 | miR-21-5p sponge | Decreased cellular growth and increased secretion of inflammatory cytokines | Downregulated in blood from patients with AKI | 53 |
| ciRs-126 | miR-126-5p sponge | Biomarker of AKI | Upregulated in blood from patients with AKI | 54 |
| ***Kidney transplantation*** | | | | |
| hsa_circ_0001334 | Unknown | Biomarker of acute TCMR | Upregulated in the urine of patients with acute TCMR | 56 |

AKI, acute kidney injury; ATF3, cyclic AMP-dependent transcription factor; Far1, fatty acyl-CoA reductase 1; PBMC, peripheral blood mononuclear cell; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; TCMR, T cell-mediated rejection.

Whether this circRNA also has a role in lupus nephritis remains to be investigated.

Another circRNA, circHLA-C, has been hypothesized to sponge miR-150 in patients with lupus nephritis[50]. CircRNA expression profiling identified 171 circRNAs that were differentially regulated in kidney biopsy samples from patients with lupus nephritis compared with healthy kidney tissue. circHLA-C was the most highly upregulated of these circRNAs and positively associated with levels of proteinuria, serum creatinine, disease activity scores and glomerular injury, suggesting that it could be a biomarker of lupus nephritis. A bioinformatics analysis identified MiR-150 as a target of circHLA-C, suggesting that the MiR-150–circHLA-C interaction might have a role in disease pathogenesis.

CircRNA expression profiling also identified 955 circRNAs that were differentially expressed (645 upregulated and 310 downregulated) in the blood of patients with membranous nephropathy compared with healthy individuals[51]. One of the most highly upregulated circRNAs in these patients was circ_101319. A circRNA–miRNA interaction network analysis suggested that circ_101319 might be associated with disease-specific signalling pathways, including MAPK, Ras and TGFβ pathways, as well as cellular senescence. circ_101319 has binding sites for members of the miR-135 family,

Enriched terms
Gene ontology term enrichment is a technique for interpreting sets of genes that makes use of the gene ontology system of classification, in which genes are assigned to a set of predefined bins on the basis of their functional characteristics.

including miR-135a and miR-135b, suggesting that it might sponge these miRs.

Further studies are required to identify the downstream mechanisms and disease-specific-impact of circRNAs in glomerular disease.

### *Acute kidney injury*

CircRNAs have also been implicated in acute kidney injury (AKI). Expression of circ-0114427 was upregulated in the tubular compartment in a mouse model of cisplatin-induced AKI and in cultured tubular cells exposed to cisplatin[52]. circ-0114427 was found to bind and sponge miR-494, thereby regulating the expression of cyclic AMP-dependent transcription factor (ATF3) and the downstream secretion of IL-6. This study provides evidence that circ-0114427 might alter the inflammatory state in drug-induced AKI.

Expression of circYAP1 was downregulated in blood samples from patients with AKI and in a HK-2 cell model of ischaemia–reperfusion injury[53]. Overexpression of circYAP1 in HK-2 cells subjected to ischaemia–reperfusion resulted in enhanced cell growth and a reduction in the secretion of inflammatory cytokines. The researchers found that circYAP1 could sponge miR-21-5p and thereby activate the PI3K–AKT–mTOR pathway. They suggest that circYAP is downregulated in response to hypoxia and/or ischaemia and that the circYAP1–miR-21-5p axis might have beneficial effects on tubular regeneration, cell proliferation and inflammation following AKI.

A global circRNA expression analysis found that a variety of circRNAs were dysregulated in the peripheral blood of patients with AKI[54]. The most highly upregulated circRNA, ciRs-126, was an independent predictor of 4-week survival among patients with AKI in the intensive care unit (lower levels were associated with higher survival rates). Bioinformatics analysis suggested that ciRs-126 might sponge miR-126-5p, which was downregulated in patients with AKI and in cultured endothelial cells exposed to hypoxia. These intriguing findings suggest an anti-angiogenic pathway involving circRNA-mediated repression of pro-angiogenic miR-126 that requires further investigation.

The findings of a study that investigated the contribution of circRNAs to the effect of sirtuin1 (SIRT1) on phenotypic changes of vascular smooth muscle cells after hindlimb ischaemia[55] might also be relevant to ischaemic AKI, although this hypothesis remains to be investigated. Following hindlimb ischaemia, SIRT1-transgenic mice showed impaired blood flow recovery, whereas SIRT1-knockout mice showed improved blood flow recovery compared with wild type mice. In addition, pharmacological suppression of SIRT1 improved blood flow recovery in SIRT1 transgenic mice. The researchers found that the circRNA cZFP609 was packaged in exosomes and impaired angiogenesis in SIRT1-transgenic mice. Moreover, addition of exosomes containing cZFP609 to cultured endothelial cells prevented the hypoxia-induced nuclear uptake of hypoxia-inducible factor 1-alpha (HIF1α) and therefore resulted in reductions in vascular endothelial growth factor A (VEGFA) expression and angiogenic functions. Consistent with these findings, silencing of cZFP609 restored blood flow in SIRT1 transgenic mice under ischaemic conditions. The concentration of cZFP609 in the blood was significantly higher in patients with atherosclerotic disease than in healthy individuals, suggesting that this circRNA might be a biomarker and progression factor in vascular disease. Further studies are needed to investigate whether cZFP609 can be therapeutically targeted to promote angiogenesis in various disease conditions that involve hypoxia and/or suppression of angiogenic signalling, including kidney diseases.

### *Kidney transplantation*

A whole-genome expression analysis identified a number of circRNAs that were dysregulated in the urine of kidney transplant recipients with acute T cell-mediated rejection[56]. In particular, hsa_circ_0001334 was significantly upregulated in these patients compared with transplant recipients without rejection and the levels of this circRNA normalized with successful anti-rejection therapy. Moreover, higher levels of hsa_circ_0001334 at the time of rejection were associated with worse graft function 1 year after transplantation. This study was the first to investigate the urinary circRNA profile of patients with acute rejection of a kidney allograft. The release pattern of urinary circRNAs in patients with kidney disease might represent a fascinating avenue of diagnostics, as the urinary circRNA profile might provide a better reflection of intrarenal changes than the blood circRNA profile. Future studies should explore the downstream mechanisms of hsa_circ_0001334 in the setting of acute T cell-mediated rejection.

## CircRNAs in complications of chronic kidney disease

Patients with chronic kidney disease (CKD) have a highly increased risk of cardiovascular disease (CVD), including atherosclerosis-related complications. As circRNAs have important roles in gene regulation and injury responses, it is unsurprising that they have been implicated in the pathogenesis of CVD[57,58]. However, CKD is an independent risk factor for CVD[59], suggesting the existence of pathogenic mechanisms distinct from those that underlie CVD in the general population. A number of circRNAs have been reported to contribute to the pathogenesis of cardiovascular complications in CKD (TABLE 2).

### *Macrovascular complications*

In CKD, hyperactivity of the renin–angiotensin–aldosterone system, dyslipidaemia, inflammation and the accumulation of uraemic toxins[60] contribute to endothelial dysfunction, which is a critical causal factor in atherogenesis[61,62]. CircRNAs have been shown to modulate inflammatory responses in endothelial cells[63] and dysregulation of circRNAs was associated with atherogenic processes (such as cell adhesion, cell activation and the immune response) in a rabbit model[64].

The first study to report an association of circRNAs with atherosclerosis in humans showed that expression of an antisense circRNA, circANRIL, correlated positively with *INK4*/*ARF* transcription and risk of

Endothelial to mesenchymal transition
(EndoMT). A process in which an endothelial cell undergoes a series of molecular events that lead to a change in phenotype towards a mesenchymal cell such as a myofibroblast or smooth muscle cell.

atherosclerotic vascular disease[65]. A subsequent study reported that cytoplasmic circANRIL binds to PES1 and thereby impairs exonuclease-mediated pre-ribosomal RNA processing and ribosome biogenesis in macrophages and vascular smooth muscle cells, leading to p53 activation and a reduction in proliferation and thereby abrogating their causal role in the formation of fatty streaks and fibrotic plaques[33].

Patients with CKD are also at an increased risk of vascular intimal and medial calcification. The extent of calcification increases with decline in glomerular function[66] and is strongly associated with cardiovascular risk[67]. Studies suggest an involvement of circRNAs in vascular calcification. For example, circSamd4a was shown to have potent anti-calcification activity in cultured vascular smooth muscle cells via sponging of specific miRNAs, including miR-125a-3p and miR-483-5p[68]. Likewise, the antioxidant activity of melatonin was shown to reduce aortic valve calcification in *Apoe*$^{-/-}$ mice via downregulation of circRIC3, which acts as a miR-204-5p sponge to positively regulate the expression of the pro-calcification gene that encodes dipeptidyl peptidase 4 (DPP4)[69]. This finding might be relevant for patients with CKD because CKD progression is associated with a decrease in melatonin levels[70] and patients on haemodialysis have reduced nocturnal melatonin secretion[71].

### *Microvascular complications*

Endothelial damage in the capillaries of the kidney medulla and accompanying vascular rarefaction are thought to contribute to progressive decline in kidney function in CKD. Several mechanisms are involved in the loss of microvascular integrity and subsequent fibrosis in AKI and CKD. In healthy physiology, capillaries are stabilized by pericytes through multiple reciprocal receptor ligand interactions; however, in diseases with microvascular complications, such as diabetes, this crosstalk is interrupted, leading to pericyte loss, leakage and impairment of microvascular perfusion[72]. Several studies suggest that circRNAs such as circRNA-ZNF532 are involved in pericyte degeneration in mice and patients with diabetic retinopathy[73]. However, no studies to date have investigated whether circRNAs have a role in pericyte degeneration in the kidney.

Endothelial to mesenchymal transition (EndoMT) is another potential mechanism of microvascular rarefaction in CKD[74]. A study that used methamphetamine-stimulated human brain microvascular endothelial

Table 2 | **Dysregulated circRNAs in cardiovascular complications of chronic kidney disease**

| CircRNA | Function | Effect | Expression | Ref. |
|---|---|---|---|---|
| ***Endothelial inflammation*** | | | | |
| circ-RELL1 | miR-6873-3p sponge | Pro-inflammatory, increases endothelial cell activation | Upregulated in endothelial cells | 63 |
| ***Atherosclerosis*** | | | | |
| circANRIL | Binds to PES1 and impairs exonuclease-mediated pre-ribosomal RNA processing and ribosome biogenesis | Anti-atherosclerotic | Upregulated in the vascular compartment | 33,65 |
| ***Vascular calcification*** | | | | |
| circSamd4a | miRNA sponge | Inhibits vascular smooth muscle cell calcification | Downregulated in vascular smooth muscle cells | 68 |
| circRIC3 | miR-204-5p sponge | Promotes vascular calcification via upregulation of DPP4 | Downregulated in the vascular compartment | 69 |
| ***Vascular integrity*** | | | | |
| cZNF532 | miR-29a-3p sponge | Decreases pericyte detachment | Upregulated in the perivascular space | 73 |
| ***Microvascular rarefaction*** | | | | |
| HECW2 | miR-30d sponge | Targeting of miR-30d leads to increased expression of ATG5 and thereby drives endoMT | Upregulated in endothelial cells | 75 |
| ***Cardiac hypertrophy*** | | | | |
| HRCR | miR-223 sponge | Sequesters miR-223, which leads to derepression of ARC, thereby inhibiting cardiac hypertrophy | Downregulated in cardiac tissue | 82 |
| circSlc8a1 | miR-133a sponge | Sequesters miR-133a, which leads to derepression of SRF, CTGF, ADRB1 and ADCY6 and protects against pressure overload-induced hypertrophy | Downregulated in cardiac tissue | 83 |

ADCY6, adenylate cyclase 6; ADRB1, beta-1 adrenergic receptor; ARC, activity-regulated cytoskeleton-associated protein; ATG5, autophagy protein 5; CTGF, connective tissue growth factor; DPP4, dipeptidyl peptidase 4; endoMT, endothelial to mesenchymal transition; PES1, pescadillo homologue 1; SRF, serum response factor.

cells and a lipopolysaccharide inflammation-induced mouse model of EndoMT in the blood–brain barrier, demonstrated that upregulation of the circRNA HECW2 leads to sponging of miR-30d and thereby derepresses the *ATG5* gene, which encodes autophagy protein 5 (ATG5), and acts as a driver of EndoMT[75]. ATG5 is a key driver of autophagy that is involved in extension of the phagophore membrane in autophagic vesicles and has been reported to have a protective role in autophagy-dependent maintenance of proximal tubule cell homeostasis in AKI[76]. Whether this mechanism involves HECW2 is not yet known.

### *Myocardial dysfunction*

CircRNAs have been reported to have important roles in ischaemic heart disease and cardiac fibrosis[77,78]. Cardiac arrest is responsible for most cardiovascular deaths in patients with kidney failure. However, left ventricular hypertrophy, often resulting from hypertension, chronic inflammation and activation of the renin–angiotensin–aldosterone system, is also a major risk factor for sudden death in patients with CKD[79]. CircRNAs have a role in cardiac development[80] and are surprisingly abundant in adult mouse and human hearts[81]. In two mouse models of cardiac hypertrophy, heart-related circRNA was markedly downregulated in the myocardium and functioned as a sponge for miR-223, thereby attenuating its hypertrophic actions[82]. circSlc8a1 has also been shown to protect against pressure overload-induced hypertrophy by functioning as an endogenous sponge for miR-133a[83]. The potential beneficial effects of these circRNAs have not yet been investigated in models of CKD-related cardiac hypertrophy.

## CircRNAs in kidney cancer

A multitude of studies have focused on the association of circRNAs with various types of human cancers[27,84]. CircRNAs have essential roles in regulating cellular metabolism and various circRNAs have been found to contribute to altered glycolysis, lipolysis and lipogenesis or oxidative phosphorylation, which may lead to improved understanding of dysfunctional cell metabolism in cancer[85]. Moreover, circRNAs in extracellular vesicles can be taken up by distant cells and affect important biological pathways in these recipient cells, potentially promoting tumour metastasis[86–88]. CircRNAs could be potential diagnostic and prognostic biomarkers and therapeutic targets for kidney cancer.

### *Oncogenic and tumour suppressor activity*

Several studies have suggested that circRNAs might have oncogenic or antitumour activity in kidney cancer, in particular through sponging of miRNAs that are critical regulators of oncogenes and tumour suppressors. Most of these studies have investigated the effects of inhibiting or overexpressing specific circRNAs in vitro on cell migration, proliferation and apoptosis, as well as potential miRNA targets (TABLE 3). Further studies are needed to confirm the relevance of these circRNAs in kidney cancer.

More extensive studies have identified potentially kidney cancer-related circRNAs and investigated the effects of modulation of these circRNAs in animal models of renal cell carcinoma (RCC). For example, circPCNXL2, circ_000926 and hsa_circ_001895 were upregulated in RCC tissue and their knockdown in murine models of RCC suppressed tumour growth, indicating the oncogenic properties of these circRNAs[89–91]. In vitro, circPCNXL2 was found to bind to miR-153 to regulate Zinc finger E-box-binding homeobox 2 (ZEB2) expression and increase cell proliferation and invasion[89]. circ_000926 could directly bind to miR-411 to upregulate its target cadherin-2 (CDH2) and mediate epithelial to mesenchymal transition[90]. In vitro studies of hsa_circ_001895 suggested that it acts as a sponge for miR-296-5p and thereby promotes transcription factor SOX12 expression and subsequent cell proliferation, invasion and migration[91].

In contrast to these oncogenic circRNAs, circ-AKT3 and circATP2B1 were demonstrated to function as tumour suppressors. Overexpression of circ-AKT3 or circATP2B1 suppressed clear cell renal cell carcinoma (ccRCC) metastasis in murine models[92,93]. circ-AKT3 binds miR-296-3p, which targets E-cadherin and thus suppresses cell migration and invasion[92]. Expression of circATP2B1 is suppressed by oestrogen receptor beta (ERbeta), which can promote the progression of ccRCC by inhibiting the tumour suppressor disabled homologue 2-interacting protein (DAB2IP). circATP2B1 sponges miR-204-3p and thereby prevents binding of this miRNA to fibronectin 1, which is important for cell invasion[93]. Taken together, these examples suggest that circRNAs have important roles in the development of kidney cancer and metastasis and are attractive candidates for future therapeutic targeting.

### *Biomarker potential*

The few studies that have explored the biomarker potential of circRNAs in kidney cancer have focused on the tissue expression of circRNAs. For example, downregulation of the circRNA cRAPGEF5 in RCC tissues correlated with increased tumour size, advanced tumour node metastasis (TNM) stage and distant metastasis, and was an independent predictor of poor overall and recurrence-free survival in patients with RCC[94].

A microarray study of ccRCC tissue identified a circRNA signature, involving circEGLN3, circNOX4 and circRHOBTB3, that may serve as a prognostic biomarker for cancer-specific, recurrence-free and overall survival[95]. This signature improved the predictive outcome accuracy of clinical models based on clinicopathological factors. In particular, expression of circEGLN3 discriminated between malignant and normal tissue with 97% accuracy. Another study also reported that upregulation of circEGLN3 was associated with shorter survival of patients with RCC[96].

In patients with ccRCC, circ-ABCB10 was upregulated in tumour tissues compared with adjacent tissues. High expression of this circRNA correlated with advanced pathological grade and TNM stage and was an independent predictor of worse overall survival in these patients[97].

Finally, mRNA expression of METTL14 was shown to negatively correlate with TNM stage and positively

Table 3 | **Dysregulated circRNAs in kidney cancer**

| CircRNA | Function | Role | Expression in RCC tissue | Study type | Refs |
|---|---|---|---|---|---|
| hsa-circ-0072309 | miR-100 sponge | Tumour suppressor; inhibits PI3K–AKT and mTOR signalling | Downregulated | In vitro | 88 |
| hsa_circ_001895 | miR-296-5p sponge | Oncogenic; promotes SOX12 expression, cell proliferation, invasion and migration; knockdown in a murine RCC model suppressed tumour growth | Upregulated | In vivo and in vitro | 91 |
| circPCNXL2 | miR-153 sponge | Oncogenic; regulates ZEB2 expression and increases cell proliferation and invasion; knockdown in a murine RCC model suppressed tumour growth | Upregulated | In vivo and in vitro | 89 |
| circATP2B1 | miR-204-3p sponge | Tumour suppressor; prevents binding of miR-204-3p to fibronectin-1, which has a role in cell invasion; suppressed by ERbeta; overexpression in a murine ccRCC model suppressed metastasis | NA | In vivo and in vitro | 93 |
| circ-AKT3 | miR-296-3p sponge | Tumour suppressor; targets E-cadherin and suppresses cell migration and invasion; overexpression in a murine ccRCC model suppressed metastasis | Downregulated | In vivo and in vitro | 92 |
| cRAPGEF5 | miR-27a sponge | Tumour suppressor; functions via derepression of TXNIP through sponging of miR-27a; downregulation correlated with increased tumour size, advanced TNM stage and distant metastasis and independently predicted poor overall and recurrence-free survival | Downregulated | Biomarker and in vitro | 94 |
| circNOX4 | Unknown | Role unknown; a circRNA signature including circNOX4, circRHOBTB3 and circEGLN3 improved the predictive survival outcome accuracy of clinical models based on clinicopathological factors | Downregulated | Biomarker | 95 |
| circRHOBTB3 | Unknown | Role unknown; a circRNA signature including circNOX4, circRHOBTB3 and circEGLN3 improved the predictive survival outcome accuracy of clinical models based on clinicopathological factors | Downregulated | Biomarker | 95 |
| circEGLN3 | miR-1299 sponge | Oncogenic; enhanced IRF7 expression, cell proliferation and migration; a circRNA signature including circNOX4, circRHOBTB3 and circEGLN3 improved the predictive survival outcome accuracy of clinical models based on clinicopathological factors | Upregulated | In vitro and biomarker | 95,96 |
| circ-ABCB10 | Unknown | Oncogenic; high levels correlated with advanced pathological grade and TNM stage and independently predicted worse overall survival in patients with ccRCC | Upregulated | Biomarker | 97 |
| hsa_circ_0035483 | miR-335 sponge | Silencing enhanced gemcitabine sensitivity in vivo, possibly via regulation of CCNB1. hsa_circ_0035483 may facilitate gemcitabine-induced autophagy | Upregulated | In vivo and in vitro | 100 |

CCNB1, cyclin B1; ccRCC, clear cell renal cell carcinoma; ERbeta, oestrogen receptor beta; IRF7, interferon regulatory factor 7; NA, not available; RCC, renal cell carcinoma; SOX12, transcription factor SOX12; TNM, tumour node metastasis; TXNIP, thioredoxin-interacting protein; ZEB2, zinc finger E-box-binding homeobox 2.

Aptamers
Oligonucleotide or peptide molecules that bind to a specific target molecule.

correlate with overall survival of patients with RCC[98]. The researchers speculated that circRNAs might act as miRNA sponges to regulate METTL14 mRNA and thereby affect the progression of RCC. They used bioinformatics tools to assess differential regulation and potential associations between circRNAs, miRNAs and METTL14 mRNA and establish a METTL14–miRNA–circRNA interaction network. They identified potential binding interactions between METTL14 and four miRNAs (miR-130a-3p, miR-130b-3p, miR-106b-5p and miR-301a-3p) as well as 24 circRNAs that potentially sponge these miRNAs. METTL14 mRNA and these circRNAs could potentially be used as biomarkers of disease progression.

Taken together, these data suggest that circRNAs are strongly associated with kidney cancer and pathological grade. Although circRNA biomarkers have not yet been shown to outperform current approaches for diagnosis and prognosis of kidney cancer, further investigation of their potential as biomarkers is warranted. Moreover, given the stability of circRNAs and their relative abundance in exosomes, investigation of the circulating circRNA profile in kidney cancer would be particularly interesting, as would investigation of their levels in other biofluids as potential non-invasive biomarkers.

### *Roles in drug resistance*

CircRNAs also have a potential role as regulators of treatment resistance in human cancers[99]. In the context of kidney cancer, hsa_circ_0035483 has been shown to facilitate gemcitabine-induced autophagy and thereby enhance the resistance of RCC to gemcitabine[100]. hsa_circ_0035483 enhanced gemcitabine resistance and thus promoted tumour growth by sponging hsa-miR-335, which led to altered cyclin B1 (CCNB1) expression, whereas silencing of hsa_circ_0035483 enhanced gemcitabine sensitivity in vivo. Furthermore, circRNAs are differentially expressed in response to cisplatin, suggesting involvement in the pathophysiology of cisplatin-induced nephrotoxicity[101]. The potential impact of radiation therapy on circRNA expression has also been investigated; irradiation of human embryonic kidney (HEK293T) cells resulted in a clear differential circRNA expression signature[102]. These data suggest possible involvement of circRNAs in treatment resistance in kidney cancer, but further studies are necessary to clarify these relationships.

## CircRNA-based therapeutic approaches

The available data suggest that circRNAs are promising therapeutic targets. However, such targeting will require tissue and/or cell specificity to avoid the development of off-target effects in distant organs. Detailed analysis and clarification of the function of the target circRNA will therefore be imperative when designing specific RNA-based therapeutics.

A potential therapeutic approach is modulation of native pathogenic circRNAs by silencing or overexpression. For example, silencing of the circRNAs mitochondrial fission and apoptosis-related circRNA, circHIPK3, cZNF609 and circFoxo3 using short hairpin RNA had beneficial effects in various disease models[103]. CircRNAs can also be silenced using the CRISPR–Cas genome editing system. An elegant study used this approach to generate a mouse model with knockout of ciRs-7 (REF.[29]). Other potential approaches to silencing circRNAs include using anti-sense oligonucleotides that bind to circRNAs via complementary base pairing and inhibit interactions with their target molecules[103] or antagonists that block the molecular interactions of the circRNAs, for example, by shielding binding sites for protein or miRNA[104].

Overexpression of circRNAs could be achieved by packaging them into extracellular vesicles that could then be used as delivery vectors[104] or by injecting an expression plasmid conjugated with colloidal gold nanoparticles, as has been demonstrated for circFoxo3 (REF.[105]).

A potential alternative strategy to therapeutic targeting of native circRNAs is the application of artificial circRNAs with designer molecular functions. Artificial circRNAs could be engineered to sponge miRNA sponges, generate circular versions of native linear RNAs with therapeutic effects, translate protein, modulate the immune system, control protein activity (by acting as aptamers, control transcription or splicing, and replicate autonomously following delivery in vivo[103]. These approaches could potentially be utilized for the future treatment of patients with kidney disease.

## Conclusions

CircRNAs have crucial roles in gene regulation at various levels, including control of miRNA and protein function[1–4]. Their biogenesis is driven by specific genomic features, back splicing and additional fine tuning by RBPs[1–3,12,13] and they mediate central biological functions via miRNA and protein sponging and protein scaffolding[3,4,29–34]. Furthermore, some cytoplasmic circRNAs have protein-coding potential[13,35].

Emerging data suggest that circRNAs have roles in the pathophysiology of kidney disease, cardiovascular complications and kidney cancer and might be promising therapeutic targets as well as potential biomarkers for these diseases. Further studies are needed to investigate the roles of circRNAs as molecular drug targets in animal models and to validate promising biomarker candidates in multicentre trials.

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### Author contributions
All authors wrote the article. J.M.L. researched the data and reviewed or edited the manuscript before submission. Correspondence regarding this Review should be sent to J.M.L. at johan.lorenzen@uzh.ch or j.m.lorenzen@gmail.com.

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The authors declare no competing interests.

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