SIRT1 and Kidney Function

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Key Words

Kidney function · Nicotinamide adenine dinucleotide · SIRT1

Abstract

Background: SIRT1 is a nicotinamide adenine dinucleotide-dependent deacetylase belonging to the class III histone deacetylases. Abundantly expressed in the kidney, especially in the renal medulla, SIRT1 is closely involved in renal physiology and pathology. **Summary:** SIRT1 targets both histone and nonhistone proteins, participates in many important signaling pathways and mediates the regulation of longevity, metabolic homeostasis, acute stress response and DNA integrity. With regard to the kidney, SIRT1 attenuates diabetic albuminuria, reduces blood pressure and related cardiovascular diseases, resists acute kidney injury, delays kidney fibrogenesis, promotes cyst formation and benefits renal ageing. **Key Messages:** This review summarizes the biology of SIRT1 and focuses on the latest studies concerning SIRT1 as a potential therapeutic target for kidney diseases.

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Introduction

In 1986, the silent information regulator 2 (Sir2) gene was isolated from budding yeast [1]. Thirteen years later, the Sir2 gene was identified as being associated with the

life span of yeast. Sinclair and Guarente [2] found that the accumulation of extrachromosomal ribosomal DNA (rDNA) circles is a major cause of ageing in yeast, which is generated by homologous recombination between rDNA repeats. Sir2 overexpression silences transcription at silent mating loci, telomeres and the rDNA and suppresses extrachromosomal rDNA circle formation to extend the life span of yeast, while deletion of Sir2 shortens the life span. This discovery was validated by a recent quantitative genetic analysis of whole genomes of different strains of yeast, which showed that, as the first quantitative trait locus, the Sir2 gene is responsible for the majority of the effects on yeast's replicative life span [3]. During this period, the antiageing effect of Sir2 and its homologs was also observed in *Drosophila*, *Caenorhabditis elegans* and mice when their expression levels are increased [4–7].

In mammals, the sirtuin family is the homolog of the Sir2 gene in yeast, consisting of at least 7 isoforms, i.e. SIRT1–SIRT7. As a commonality of the sirtuin family, the 7 isoforms have the same 275-amino-acid-sized catalytic core region and a diverse subcellular localization. SIRT1, SIRT6 and SIRT7 are mainly found in the nucleus, and SIRT2 is in the cytoplasm, while SIRT3, SIRT4 and SIRT5 are localized in the mitochondria [8]. SIRT1 is the most extensively studied family member.

The dietary intervention of caloric restriction (CR) was first experimented with in rats in 1934 [9], and it showed a positive impact by increasing their life span

without malnutrition. Afterwards, the effect of delaying ageing and extending the life span were demonstrated in diverse species including primates [10, 11]. In addition to this, CR benefits patients with a wide range of age-related diseases such as diabetes, cancer, cardiovascular disease, neurodegenerative diseases and age-associated renal injury [10, 12–14]. Interestingly, SIRT1 overexpression and CR share phenotypes of antiageing and antiageing-related diseases [15, 16]. The study of yeast helps to link the two together. When the glucose concentration is reduced from 2 to 0.5%, the average life span of yeast (the number of generations) increases from 21.1 to 26 [17]. A similar life extension phenomenon cannot be repeated in the Sir2 mutant strain, suggesting that CR-induced longevity is dependent on activation of the Sir2 protein. As for mammals, in several studies [18, 19] SIRT1 was proven to be the key regulator of physiological adaption when subjected to different diets including fasting, CR and a high-fat diet. CR, the mechanism of which remains complicated and not yet fully understood, is proposed to benefit rodents at least in part by activating SIRT1; in vivo effects of SIRT1-activating compounds further support this theory. Resveratrol (*trans*-3,5,4'-trihydroxystilbene) and its analogs are SIRT1 activators and show life-extending, anti-inflammatory, antioxidant, antidiabetic and cancer-preventive effects [20].

The first clue about how SIRT1 performs its function also comes from a study on yeast. Overexpression of yeast Sir2 proteins, together with the presence of nicotinamide adenine dinucleotide (NAD⁺), promotes global deacetylation of histones, especially lysines 9 and 14 of histone H3 and lysine 16 of histone H4, which are acetylated in active chromatin and hypoacetylated in silenced chromatin [21]. This enzyme activity of histone deacetylases categorizes sirtuins into the class III family of histone deacetylase enzymes, for it requires NAD⁺ as a cofactor instead of zinc in class I and II histone deacetylases. Therefore, sirtuins are able to monitor metabolic homeostasis via variation of the NAD⁺ level, which is sensitive to the intracellular state of energy and redox change. In order to catalyze the conversion of an acetylated substrate to a deacetylated substrate, NAD⁺ is firstly cleaved into nicotinamide and *O*-acetyl-ADP-ribose, the latter of which will covalently attach to the acetyl moiety of the substrate. Then, hydrolysis of the acetyl-lysine bond liberates *O*-acetyl-ADP-ribose and makes itself biologically active [22]. The deacetylation reaction yields nicotinamide, which in turn serves as an inhibitor to the reaction itself and becomes a salvage pathway in which NAD⁺ is resynthesized from nicotinamide [8].

In the sirtuin family, SIRT1, SIRT2 and SIRT3 are NAD⁺-dependent deacetylases. In addition to its deacetylase function, SIRT1 also has mono-ADP-ribosyltransferase activity (transfer of ADP-ribose to other proteins), which is of minor importance for its biologic function, while SIRT4 primarily functions as a mono-ADP-ribosyltransferase, SIRT5 possesses desuccinylase and demalonylase activities [23], and SIRT6 can remove long-chain fatty acids from selected substrate proteins [24].

Another important characteristic of sirtuins is that they target a variety of transcriptional factors for deacetylation besides histone. Lysine acetylation activity is a dynamic, reversible and evolutionarily conserved protein posttranslational modification. A recent proteomic study [25] highlights the significant role of SIRT1 in regulating diverse substrates and cellular pathways. A total number of 4,623 lysine acetylation sites in 1,800 proteins were identified and quantified via HPLC-MS/MS analysis using mouse embryonic fibroblasts isolated from wild-type and SIRT1 knockout mice, of which 485 lysine acetylation sites are enhanced by more than 100% after SIRT1 knockout. Interestingly, not all of these sites are necessarily direct SIRT1 targets, because a number of acetyltransferases and major acetyltransferase complexes such as Kat5 (Tip60), Kat8 (Myst1) and p300 are targeted and regulated by SIRT1-mediated deacetylation. With regard to the direct substrates deacetylated by SIRT1, acetylated modification of these transcription factors and transcriptional coregulatory proteins by SIRT1 may have an influence on them in different ways, including altering their stability, activity, subcellular localization, DNA-binding ability and protein-protein interactions [26, 27]. Notably, the results of this modification by SIRT1 may vary, depending on the kind of stimuli, the stimulated protein and its acetylation site. Take p53 as an example. In response to DNA damage, SIRT1 binds and deacetylates the p53 protein with specificity for its C-terminal Lys382 residue [28]. Deacetylated p53 results in nuclear translocation and reduced DNA-binding ability, which lead to either growth arrest or apoptosis [29].

Regulation of SIRT1 Activity

Since SIRT1 is such an essential metabolic sensor, its activity is regulated dynamically in order to allow for adaptations to any alteration to the cellular metabolic state. Therefore, nutritional, hormonal and environmental signals, as well as the NAD⁺ level and the SIRT1-interacting proteins responding to those signals, compose the regulation network of SIRT1.

With a high-fat and high-glucose diet, SIRT1 expression is decreased, while during starvation and nutrient deprivation, SIRT1 expression is increased [30]. In experiments of acute nutrient withdrawal, the forkhead transcription factor Foxo3a is activated and is in physical interaction with p53 [31]. Being mediated through two p53 binding sites present in the SIRT1 promoter, Foxo3a stimulates SIRT1 transcription. SIRT1 expression cannot be induced in either Foxo3a knockdown cells or starved p53-deficient mice. Thus, SIRT1 is regulated in a nutrient-sensing pathway involving the reciprocal action of relevant proteins.

During stress response, SIRT1 links chromatin dynamics/gene expression to environmental stimuli. SIRT1 induced by acute stress ensures genome integrity [32]. SIRT1 mediates heterochromatin formation by deacetylating histone polypeptides with a preference for histone H4 lysine 16 (H4-K16Ac) and H3 lysine 9 (H3-K9Ac), followed by recruitment and deacetylation of histone H1 at lysine 26. This facultative heterochromatin results in a mark restricting silenced chromatin with hypomethylation of H3-K79 [33]. Also, SIRT1 enhances methyltransferase activity. Following DNA damage, SIRT1 may generate aberrant methylation of the CpG islands in promoters, promoting heritable gene silencing [34]. However, the endogenous mechanisms that regulate the level and activity of SIRT1 are not clear. There are studies suggesting both posttranscriptional and posttranslational modifications of SIRT1. The RNA-binding protein HuR, regulating the stability of many target mRNAs, associated with the 3' untranslated region of the mRNA encoding SIRT1, stabilizes the SIRT1 mRNA and increases SIRT1 expression levels, thus functioning in a posttranscriptional way [35]. Sumoylation of SIRT1 at Lys 734 increased its deacetylase activity. Conversely, mutation of SIRT1 at Lys 734 or desumoylation by SENP1, a nuclear desumoylase, reduces its deacetylase activity. Stress-inducing agents promote the association of SIRT1 with SENP1, and cells depleted of SENP1 (but not of SENP1 and SIRT1) are more resistant to stress-induced apoptosis than control cells [36]. Another posttranslational modification is phosphorylation. Mass spectrometry shows that the cell cycle-dependent kinase cyclin B/Cdk1 forms a complex with and phosphorylates SIRT1 at threonine 530 and serine 540 [37].

Given the fact that NAD⁺ serves as the core of the SIRT1 deacetylase enzymatic reaction, NAD biosynthesis critically regulates its activity [38]. NAD⁺ is generated from tryptophan and vitamin B₃, which is also known as nicotinic acid or nicotinamide, from dietary intake de

novo [39]. Interestingly, nicotinamide, a by-product of SIRT1 and another enzyme-mediated reaction which NAD⁺ participates in as a cofactor, serves as an endogenous inhibitor of SIRT1 and is also involved in the resynthesis of NAD⁺ in the salvage pathway. This resynthesis is of great significance, because it ensures a SIRT1-mediated reaction via lowering the concentration of nicotinamide and raising NAD⁺ levels. First, nicotinamide is converted into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (Nampt). Subsequently, NMN adenylyltransferase (Nmnat) regenerates NAD⁺ from NMN. Nampt is the rate-limiting enzyme of the NAD⁺ salvage re-cycle and a link to the circadian clock cycle. CLOCK:BMAL1 (circadian locomotor output cycles kaput-brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1) is physically and specifically associated with the E-boxes on the Nampt promoter, and active Nampt induces SIRT1 by regenerating NAD⁺ [38]. Moreover, CLOCK has an intrinsic acetyltransferase activity, which enables circadian chromatin remodeling by acetylating histones and nonhistone proteins, and SIRT1 acts as the histone deacetylase that counterbalances the histone acetyltransferase function of CLOCK. Thus, a loop of the cellular metabolism and the circadian clock emerges, and any attacks on the loop may have an impact on SIRT1. As a result, lack of NAD⁺ decreases SIRT1 activity, and possible reasons for NAD⁺ shortage include serious nutrition insufficiency, excessive consumption by activated poly-ADP-ribose polymerases in stress response and disturbed recycling from nicotinamide in the salvage pathway.

Relevance of SIRT1 to Metabolic Kidney Diseases

SIRT1 has been found to be an essential factor in the regulation of systemic metabolic homeostasis [40] as well as a metabolic sensor in the modulation of gene expression in response to changes in cellular energy states in multiple organs including the kidney [41]. Increased SIRT1 activity by either pharmacologic or genetic means can prolong the life of obese animals and significantly improves their metabolic parameters such as glucose tolerance, fasting blood glucose levels and insulin resistance [42, 43]. Since the kidney is very likely to be attacked in metabolic disorders such as diabetes mellitus, and diabetes is associated with reduced SIRT1 expression in the kidney, it is likely that the kidney may benefit from SIRT1 activation. This is supported by recent studies. Hasegawa et al. [44] used mouse models of diabetes mellitus to show

that decreased SIRT1 in proximal tubules represents the onset of diabetic nephropathy. In their experiment, albuminuria could be prevented by overexpressing SIRT1 in the proximal tubules of a transgenic mouse model, while in the proximal tubule-specific SIRT1 conditional knockout mouse model, streptozotocin-induced albuminuria showed no signs of improvement. This is due to the cross talk between SIRT1 and claudin-1. In the diabetic milieu, reduced expression of SIRT1 causes downregulation of intracellular-type Namp1, presumably by direct transcriptional mechanisms, which leads to a reduction of the levels of NMN secreted from tubular cells. As a result, the basal level of NMN around podocytes will no longer be maintained, so that SIRT1 expression is decreased and fails to modulate claudin-1 epigenetically. Activated claudin-1 in podocytes impairs the glomerular barrier function through downregulating synaptopodin or podocin expression and induces albuminuria [44]. Podocyte SIRT1 is also involved in the pathophysiology of diabetic nephropathy. Podocyte-specific SIRT1 knockout mice show increased urinary albumin excretion in diabetes [Guan and Hao, unpubl. data]. It is suggested that in the diabetic kidney, SIRT1 in the podocytes functions as a deacetylase and inactivates the p65 subunit of NF- κ B and STAT3 in order to protect the podocytes from injury [45]. Also, our laboratory reported that resveratrol, a SIRT1 activator, attenuated diabetic kidney injury via modulating abnormal angiogenesis in a type 1 diabetic rat model [46].

Hypertension is frequently clustered with obesity, hyperlipidemia and hyperinsulinemia, collectively termed the 'metabolic syndrome', because the regulation of systemic blood pressure is strongly associated with energy metabolism. It has been documented that reduced caloric dietary intake decreases arterial blood pressure in mildly hypertensive patients or reduces the number of antihypertensive drugs required to control hypertension. Recent studies have suggested that SIRT1 is involved in blood pressure through the regulation of renal sodium reabsorption in the collecting duct and the regulation of vascular tone.

Aldosterone increases renal tubular Na⁺ absorption by enhancing the activity of the epithelial Na⁺ channel α -subunit (α -ENaC) expressed in the apical membrane of principal cells of the collecting duct. Zhang et al. [47] explored that a complex containing the histone H3K79 methyltransferase disruptor of telomeric silencing-1 (Dot1) is associated with and represses the α -ENaC promoter in murine inner medullary collecting duct cells, and SIRT1 overexpression can inhibit basal α -ENaC

mRNA expression and α -ENaC promoter activity by functionally and physically interacting with Dot1 to enhance the distributive activity of Dot1 on H3K79 methylation, surprisingly in a deacetylase-independent manner, and independent of mineralocorticoid receptor signaling as well. Since the effects of SIRT1 on α -ENaC transcription are independent of its enzymatic activity, changes in nicotinamide levels or the use of SIRT1 activators are unlikely to modulate sodium reabsorption in the collecting duct [47].

Several studies have demonstrated that SIRT1 promotes endothelium-dependent vasodilation by targeting endothelial nitric oxide synthase (eNOS) for deacetylation [48, 49]. SIRT1 and eNOS colocalize and coprecipitate in endothelial cells, and SIRT1 deacetylates eNOS, stimulating eNOS activity and increasing endothelial nitric oxide (NO). The SIRT1-induced increase in endothelial NO is mediated through lysines 496 and 506 in the calmodulin-binding domain of eNOS. Inhibition of SIRT1 in the endothelium of arteries inhibits endothelium-dependent vasodilation and decreases bioavailable NO. Moreover, vascular smooth muscle cell-specific SIRT1 transgenic mice prevented the increase in systolic blood pressure caused by angiotensin II infusion without vascular remodeling in murine thoracic and renal aortas [50]. SIRT1 overexpression significantly inhibited reactive oxygen species (ROS) generation, vascular inflammation and collagen synthesis in arterial walls and decreases the angiotensin II-increased binding of NF- κ B on its specific binding sites on the TGF- β ₁ promoter. Ichiki et al. [51] and Miyazaki et al. [52] likewise showed that administration of resveratrol, a SIRT1 activator, not only suppresses AT1R expression in the aorta but also significantly blunts angiotensin II-induced hypertension in mice.

Acute Kidney Injury

Renal tubules are easy to be affected by acute hemodynamic changes or toxins, which may lead to acute kidney injury (AKI). In a cisplatin-induced AKI model, the effect of SIRT1 was investigated by using transgenic mice overexpressing SIRT1 specifically in proximal tubules. SIRT1-overexpressing mice can preserve both the number of peroxisomes and their catalase activity, which is severely decreased by cisplatin; thus, SIRT1 exerts protective action in tubular cell damage and kidney injury [53]. Elevated ROS levels within mitochondria are deleterious to both the mitochondria and mitochondrion-rich tubular cells, and damaged mitochondria in turn produce more

ROS, which leads to cell apoptosis and necrosis in AKI. This excessive oxidative stress and mitochondrial dysfunction can be altered by SIRT1 activation. SRT1720, a specific SIRT1 activator, can deacetylate and activate peroxisome proliferator-activated receptor- γ coactivator 1 α in order to expedite the recovery of mitochondrial protein expression and function, as well as normal mitochondrial biogenesis [54, 55]. Also, high SIRT1 expression contributes to cell regeneration following ischemia/reperfusion-induced AKI. SIRT1 heterozygotes are more susceptible to ischemia/reperfusion injury and hold a significantly higher level of the cyclin-dependent kinase (CDK) inhibitor p21. p21, which is downstream of p53, binds to and inhibits the activity of cyclin-CDK2 or cyclin-CDK4 complexes and thus functions as a regulator of cell cycle progression at G₁. Either SIRT1 or SIRT1-induced lower p53 levels might suppress p21 and result in increased cell proliferation [56].

Renal Fibrogenesis

Renal fibrogenesis is the common outcome of various diseases damaging the kidney, and it leads to end-stage renal failure. The antifibrotic function of SIRT1 has been demonstrated using several kinds of renal fibrosis animal model. In the unilateral ureteral obstruction model, SIRT1, abundantly expressed in murine renal medullary interstitial cells, can attenuate oxidative stress-induced COX2 expression to rescue the interstitium from inflammation and fibrogenesis [57].

TGF- β can induce extracellular matrix production and promote fibrogenesis, which signals through the heteromeric complex of TGF- β type I receptor with TGF- β type II receptor to activate and assemble Smad2, Smad3 and Smad4 into heteromeric complexes that translocate into the nucleus [58, 59]. Thus, these complexes in the nucleus can interact with various transcription factors and regulate the expression of TGF- β target genes [60]. In the obstructed kidney, Smad3 acetylation, as well as its phosphorylation, is an early response in the induction of interstitial fibrosis. Resveratrol can prevent Smad3 acetylation, but not phosphorylation, resulting in less interstitial fibrosis [61]. The underlying mechanism was elucidated by Huang et al. [62], using a 5/6 nephrectomy model: deacetylation of Smad3 directly induced by SIRT1 activation can attenuate renal damage by inhibiting TGF- β -Smad signaling. SIRT1 heterozygotes suffer from worse kidney function and end up with more serious kidney fibrosis. Simic et al. [63] examined the progression from AKI to chronic

kidney disease and reached a similar conclusion by performing the ischemia/reperfusion procedure on kidney-specific knockout mice and kidney-specific transgenic mice. The kidney-specific knockout mice subsequently showed worse kidney function and more kidney fibrosis, whereas the kidney-specific transgenic mice showed improvement. Furthermore, endothelial cell-specific SIRT1 knockout mice have impaired angiogenesis, reduced matrix activity and retention of the profibrotic cleavage substrate tissue transglutaminase as well as endoglin-accompanied MMP-14 suppression, and are susceptible to folic acid-induced kidney injury. Restoration of MMP-14 expression in SIRT1-depleted mice improved the angiogenic and matrix functions of the endothelium, prevented renal dysfunction and attenuated nephrosclerosis [64]. So far, SIRT1 seems to be a promising target of intervention for delaying the development of nephrosclerosis.

Autosomal-Dominant Polycystic Kidney Disease

As mentioned before, SIRT1 induces kidney recovery from external stress such as ischemia/reperfusion injury by inducing renal epithelial cell proliferation and reducing its apoptosis. Recently, Li and colleagues [65] suggested that, compared to mice with single conditional knockout of Pkd1, a classic autosomal dominant polycystic kidney disease animal model, with double conditional knockout of Pkd1 and SIRT1, showed delayed renal cyst formation in postnatal murine kidneys. Further, inhibiting SIRT1 with nicotinamide or the SIRT1-specific inhibitor EX-527 delayed cyst formation in Pkd1-null murine embryonic kidneys and in Pkd1-conditional knockout postnatal kidneys. This finding is in accordance with previous studies demonstrating that SIRT1 can promote the recovery of the kidney from injury, because autosomal dominant polycystic kidney disease is a disease with continuously abnormal cyst formation; in either damaged tubules or cysts, mechanistically, SIRT1 promotes growth of the epithelium by deacetylation and inactivation of Rb and p53.

Renal Ageing

CR consists of established dietary interventions that have been shown to increase both the median and the maximum life spans of a variety of species, including yeast, fish and mammals [17, 66]. SIRT1 activity is suggested to be increased by CR, and SIRT1 is one of the mol-

ecules that mediate the beneficial effects of CR. SIRT1-overexpressing transgenic mice show a phenotype that resembles mice under CR [15], and SIRT1-deficient mice failed to show life span prolongation under CR [67]. Kume et al. [68] demonstrated that the kidney also benefits from CR. Ageing leads to significantly decreased Sirt1 expression and enhanced PI3K, which suppress both autophagy and cell cycle arrest, resulting in the accumulation of oxidative stress and subsequent apoptosis through acetylated Foxo3-mediated Bim expression, whereas a 12-month CR diet rescues SIRT1 downregulation in the aged kidney. Preserved SIRT1 function promotes cell adaptation to hypoxia through deacetylated Foxo3-enhanced Bnip3-mediated autophagy and p27Kip1 expression. Our research team is also interested in renal ageing. We find that younger mice are more resistant to both ischemia/reperfusion [56] and cisplatin-induced kidney injury [Guan et al., unpubl. data]. Both SIRT1 expression and the NAD⁺ cycle decline as age advances. Losing one allele of SIRT1 makes SIRT1 heterozygotes similarly vulnerable to injury as aged mice. Specific agonists of SIRT1 as well as pharmaceutically re-filled NAD⁺ can activate SIRT1 to lower the risk of kidney injury following acute stress. The responsible mechanism involves the effect of SIRT1 on cell survival and mitochondrial function.

Clinical Relevance of SIRT1

Few studies show SIRT1 to be involved in human kidney disease despite the fact that SIRT1 plays an important role in the pathogenesis of kidney disease in mammals. This might be due to difficulty acquiring human kidney tissue specimens. Hasegawa et al. [44] showed by immunohistochemistry of human kidney biopsy sections that lack of glomerular SIRT1 epigenetically increased the expression of the tight junction protein claudin-1, resulting in serious proteinuria.

A Japanese case-control study on type 2 diabetes [69] identified 4 single nucleotide polymorphisms (SNPs) within the SIRT1 gene which have been nominally associated with susceptibility to diabetic nephropathy; a haplotype consisting of the 11 SNPs in the SIRT1 locus had a stronger association. Another Japanese study [70] enrolled 219 hemodialysis patients and 803 control subjects and revealed that the SIRT1 polymorphisms rs7069102 and rs2273773 are associated with abnormal cholesterol metabolism and coronary artery calcification, respectively, in Japanese HD patients; whether they have an impact on survival is not yet clear.

Since SIRT1 is an antiageing molecule and has a protective effect on multiple organs such as the heart, neurons and kidneys, the scientific community keeps seeking its activator. SRT1720, a synthetic SIRT1-activating compound, has been proven to attenuate damage induced by ischemia/reperfusion and obstructive kidney disease [56, 57]. Resveratrol, a natural antioxidant present in red wine, is a more widely recognized candidate for activating SIRT1. Resveratrol extends the life span of yeast, worms and flies [71], protects rodent kidneys from fibrogenesis and diabetic nephropathy [46, 62] and improves metabolic parameters in aged mice and obese humans [42, 72]. A molecule as small as 228 Da in size, resveratrol is likely to regulate the activity of other cellular proteins in addition to SIRT1. For example, resveratrol binds to a hydrophobic pocket of F1-ATPase and inhibits this key enzyme in mitochondrial respiration [73], which may indirectly activate AMP-activated protein kinase. Nevertheless, a recent experiment led by Sinclair and colleagues [74] ends the debate on whether resveratrol directly activates SIRT1 and supports the model that some of the biological effects of resveratrol and SIRT1-activating compounds are strictly dependent on SIRT1. Many clinical trials of resveratrol have been carried out or are going on at the moment. Due to the limitations of resveratrol regarding its bioavailability, pharmacokinetics and target specificity, the results are expected to be diverse. So far, at least three human studies have shown protective effects of resveratrol by systolic blood pressure reduction and improved insulin sensitivity [72, 75, 76].

Once again, the SIRT1 activator is back on stage, and further, exquisitely designed studies are required to elucidate its role in a pharmacological approach to renal protection and healthy ageing.

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Disclosure Statement

The authors declare no conflicts of interest.

References

- Ivy JM, Klar AJ, Hicks JB: Cloning and characterization of four SIR genes of *Saccharomyces cerevisiae*. *Mol Cell Biol* 1986;6:688–702.
- Sinclair DA, Guarente L: Extrachromosomal rDNA circles – a cause of aging in yeast. *Cell* 1997;91:1033–1042.
- Stumpferl SW, Brand SE, Jiang JC, Korona B, Tiwari A, Dai J, Seo JG, Jazwinski SM: Natural genetic variation in yeast longevity. *Genome Res* 2012;22:1963–1973.
- Tissenbaum HA, Guarente L: Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*. *Nature* 2001;410:227–230.
- Rogina B, Helfand SL: Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci USA* 2004;101:15998–16003.
- Viswanathan M, Guarente L: Regulation of *Caenorhabditis elegans* lifespan by *sir-2.1* transgenes. *Nature* 2011;477:E1–E2.
- Banerjee KK, Ayyub C, Ali SZ, Mandot V, Prasad NG, Kolthur-Seetharam U: dSir2 in the adult fat body, but not in muscles, regulates life span in a diet-dependent manner. *Cell Rep* 2012;2:1485–1491.
- Wakino S, Hasegawa K, Itoh H: Sirtuin and metabolic kidney disease. *Kidney Int* 2015, Epub ahead of print.
- McCay CM, Crowell MF: Prolonging the life span. *Sci Mon* 1934;39:405–414.
- Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, et al: Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 2009;325:201–204.
- Barger JL, Walford RL, Weindruch R: The retardation of aging by caloric restriction: its significance in the transgenic era. *Exp Gerontol* 2003;38:1343–1351.
- Stern JS, Gades MD, Wheelon CM, Borchers AT: Calorie restriction in obesity: prevention of kidney disease in rodents. *J Nutr* 2001;131:913s–917s.
- Pfluger PT, Herranz D, Velasco-Miguel S, Serrano M, Tschöp MH: Sirt1 protects against high-fat diet-induced metabolic damage. *Proc Natl Acad Sci USA* 2008;105:9793–9798.
- Joseph J, Cole G, Head E, Ingram D: Nutrition, brain aging, and neurodegeneration. *J Neurosci* 2009;29:12795–12801.
- Bordone L, Cohen D, Robinson A, Motta MC, van Veen E, Czopik A, Steele AD, Crowe H, Marmor S, Luo J, et al: SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell* 2007;6:759–767.
- Chen D, Steele AD, Lindquist S, Guarente L: Increase in activity during calorie restriction requires Sirt1. *Science* 2005;310:1641.
- Lin SJ, Defossez PA, Guarente L: Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 2000;289:2126–2128.
- Rodgers JT, Puigserver P: Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. *Proc Natl Acad Sci USA* 2007;104:12861–12866.
- Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, Leid M, McBurney MW, Guarente L: Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR- γ . *Nature* 2004;429:771–776.
- Yang H, Baur JA, Chen A, Miller C, Adams JK, Kisieleski A, Howitz KT, Zipkin RE, Sinclair DA: Design and synthesis of compounds that extend yeast replicative lifespan. *Aging Cell* 2007;6:35–43.
- Imai S, Armstrong CM, Kaerberlein M, Guarente L: Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 2000;403:795–800.
- Sinclair DA, Guarente L: Small-molecule allosteric activators of sirtuins. *Annu Rev Pharmacol Toxicol* 2014;54:363–380.
- Peng C, Lu Z, Xie Z, Cheng Z, Chen Y, Tan M, Luo H, Zhang Y, He W, Yang K, et al: The first identification of lysine malonylation substrates and its regulatory enzyme. *Mol Cell Proteomics* 2011;10:M111.012658.
- Jiang H, Khan S, Wang Y, Charron G, He B, Sebastian C, Du J, Kim R, Ge E, Mostoslavsky R, et al: SIRT6 regulates TNF- α secretion through hydrolysis of long-chain fatty acyl lysine. *Nature* 2013;496:110–113.
- Chen Y, Zhao W, Yang JS, Cheng Z, Luo H, Lu Z, Tan M, Gu W, Zhao Y: Quantitative acetylome analysis reveals the roles of SIRT1 in regulating diverse substrates and cellular pathways. *Mol Cell Proteomics* 2012;11:1048–1062.
- Sadoul K, Boyault C, Pabion M, Khochbin S: Regulation of protein turnover by acetyltransferases and deacetylases. *Biochimie* 2008;90:306–312.
- Wang C, Tian L, Popov VM, Pestell RG: Acetylation and nuclear receptor action. *J Steroid Biochem Mol Biol* 2011;123:91–100.
- Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, Guarente L, Weinberg RA: *hSIR2^{SIRT1}* functions as an NAD-dependent p53 deacetylase. *Cell* 2001;107:149–159.
- Gonfloni S, Iannizzotto V, Maiani E, Bellusci G, Ciccone S, Diederich M: p53 and Sirt1: routes of metabolism and genome stability. *Biochem Pharmacol* 2014;92:149–156.
- Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P: Nutrient control of glucose homeostasis through a complex of PGC-1 α and SIRT1. *Nature* 2005;434:113–118.
- Nemoto S, Fergusson MM, Finkel T: Nutrient availability regulates SIRT1 through a forkhead-dependent pathway. *Science* 2004;306:2105–2108.
- Bosch-Presegué L, Vaquero A: Sirtuins in stress response: guardians of the genome. *OncoGene* 2014;33:3764–3775.
- Vaquero A, Scher M, Lee D, Erdjument-Bromage H, Tempst P, Reinberg D: Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell* 2004;16:93–105.
- O'Hagan HM, Mohammad HP, Baylin SB: Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island. *PLoS Genet* 2008;4:e1000155.
- Abdelmohsen K, Pullmann R Jr, Lal A, Kim HH, Galban S, Yang X, Blethrow JD, Walker M, Shubert J, Gillespie DA, et al: Phosphorylation of HuR by Chk2 regulates SIRT1 expression. *Mol Cell* 2007;25:543–557.
- Yang Y, Fu W, Chen J, Olashaw N, Zhang X, Nicosia SV, Bhalla K, Bai W: SIRT1 sumoylation regulates its deacetylase activity and cellular response to genotoxic stress. *Nat Cell Biol* 2007;9:1253–1262.
- Sasaki T, Maier B, Koclega KD, Chruszcz M, Gluba W, Stukenberg PT, Minor W, Scoble H: Phosphorylation regulates SIRT1 function. *PLoS One* 2008;3:e4020.
- Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P: Circadian control of the NAD⁺ salvage pathway by CLOCK-SIRT1. *Science* 2009;324:654–657.
- Imai S: Dissecting systemic control of metabolism and aging in the NAD world: the importance of SIRT1 and NAMPT-mediated NAD biosynthesis. *FEBS Lett* 2011;585:1657–1662.
- Purushotham A, Xu Q, Li X: Systemic SIRT1 insufficiency results in disruption of energy homeostasis and steroid hormone metabolism upon high-fat-diet feeding. *FASEB J* 2012;26:656–667.
- Li X, Kazgan N: Mammalian sirtuins and energy metabolism. *Int J Biol Sci* 2011;7:575–587.
- Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, et al: Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006;444:337–342.
- Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, Vu CB, et al: Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* 2007;450:712–716.
- Hasegawa K, Wakino S, Simic P, Sakamaki Y, Minakuchi H, Fujimura K, Hosoya K, Komatsu M, Kaneko Y, Kanda T, et al: Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. *Nat Med* 2013;19:1496–1504.
- Liu R, Zhong Y, Li X, Chen H, Jim B, Zhou MM, Chuang PY, He JC: Role of transcription factor acetylation in diabetic kidney disease. *Diabetes* 2014;63:2440–2453.
- Wen D, Huang X, Zhang M, Zhang L, Chen J, Gu Y, Hao CM: Resveratrol attenuates diabetic nephropathy via modulating angiogenesis. *PLoS One* 2013;8:e82336.

- 47 Zhang D, Li S, Cruz P, Kone BC: Sirtuin 1 functionally and physically interacts with disruptor of telomeric silencing-1 to regulate α -ENaC transcription in collecting duct. *J Biol Chem* 2009;284:20917–20926.
- 48 Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB, DeRico J, Kasuno K, Irani K: SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 2007;104:14855–14860.
- 49 Jung SB, Kwon SK, Kwon M, Nagar H, Jeon BH, Irani K, Yoon SH, Kim CS: Docosahexaenoic acid improves vascular function via up-regulation of SIRT1 expression in endothelial cells. *Biochem Biophys Res Commun* 2013;437:114–119.
- 50 Gao P, Xu TT, Lu J, Li L, Xu J, Hao DL, Chen HZ, Liu DP: Overexpression of SIRT1 in vascular smooth muscle cells attenuates angiotensin II-induced vascular remodeling and hypertension in mice. *J Mol Med (Berl)* 2014;92:347–357.
- 51 Ichiki T, Miyazaki R, Kamiharaguchi A, Hashimoto T, Matsuura H, Kitamoto S, Tokunou T, Sunagawa K: Resveratrol attenuates angiotensin II-induced senescence of vascular smooth muscle cells. *Regul Pept* 2012;177:35–39.
- 52 Miyazaki R, Ichiki T, Hashimoto T, Inanaga K, Imayama I, Sadoshima J, Sunagawa K: SIRT1, a longevity gene, downregulates angiotensin II type 1 receptor expression in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2008;28:1263–1269.
- 53 Hasegawa K, Wakino S, Yoshioka K, Tatemasu S, Hara Y, Minakuchi H, Sueyasu K, Washida N, Tokuyama H, Tzukerman M, et al: Kidney-specific overexpression of Sirt1 protects against acute kidney injury by retaining peroxisome function. *J Biol Chem* 2010;285:13045–13056.
- 54 Funk JA, Schnellmann RG: Accelerated recovery of renal mitochondrial and tubule homeostasis with SIRT1/PGC-1 α activation following ischemia-reperfusion injury. *Toxicol Appl Pharmacol* 2013;273:345–354.
- 55 Wang H, Guan Y, Karamercan MA, Ye L, Bhatti T, Becker LB, Baur JA, Sims CA: Resveratrol rescues kidney mitochondrial function following hemorrhagic shock. *Shock* 2015;44:173–180.
- 56 Fan H, Yang HC, You L, Wang YY, He WJ, Hao CM: The histone deacetylase, SIRT1, contributes to the resistance of young mice to ischemia/reperfusion-induced acute kidney injury. *Kidney Int* 2013;83:404–413.
- 57 He W, Wang Y, Zhang MZ, You L, Davis LS, Fan H, Yang HC, Fogo AB, Zent R, Harris RC, et al: Sirt1 activation protects the mouse renal medulla from oxidative injury. *J Clin Invest* 2010;120:1056–1068.
- 58 Böttinger EP, Bitzer M: TGF- β signaling in renal disease. *J Am Soc Nephrol* 2002;13:2600–2610.
- 59 Roberts AB, Russo A, Felici A, Flanders KC: Smad3: a key player in pathogenetic mechanisms dependent on TGF- β . *Ann NY Acad Sci* 2003;995:1–10.
- 60 Roberts AB, Tian F, Byfield SD, Stuelten C, Ooshima A, Saika S, Flanders KC: Smad3 is key to TGF- β -mediated epithelial-to-mesenchymal transition, fibrosis, tumor suppression and metastasis. *Cytokine Growth Factor Rev* 2006;17:19–27.
- 61 Li J, Qu X, Ricardo SD, Bertram JF, Nikolic-Paterson DJ: Resveratrol inhibits renal fibrosis in the obstructed kidney: potential role in deacetylation of Smad3. *Am J Pathol* 2010;177:1065–1071.
- 62 Huang XZ, Wen D, Zhang M, Xie Q, Ma L, Guan Y, Ren Y, Chen J, Hao CM: Sirt1 activation ameliorates renal fibrosis by inhibiting the TGF- β /Smad3 pathway. *J Cell Biochem* 2014;115:996–1005.
- 63 Simic P, Williams EO, Bell EL, Gong JJ, Bonkowski M, Guarente L: SIRT1 suppresses the epithelial-to-mesenchymal transition in cancer metastasis and organ fibrosis. *Cell Rep* 2013;3:1175–1186.
- 64 Vasko R, Xavier S, Chen J, Lin CH, Ratliff B, Rabadi M, Maizel J, Tanokuchi R, Zhang F, Cao J, et al: Endothelial sirtuin 1 deficiency perpetrates nephrosclerosis through down-regulation of matrix metalloproteinase-14: relevance to fibrosis of vascular senescence. *J Am Soc Nephrol* 2014;25:276–291.
- 65 Zhou X, Fan LX, Sweeney WE Jr, Denu JM, Avner ED, Li X: Sirtuin 1 inhibition delays cyst formation in autosomal-dominant polycystic kidney disease. *J Clin Invest* 2013;123:3084–3098.
- 66 Weindruch R, Naylor PH, Goldstein AL, Walford RL: Influences of aging and dietary restriction on serum thymosin α_1 levels in mice. *J Gerontol* 1988;43:B40–B42.
- 67 Boily G, Seifert EL, Bevilacqua L, He XH, Saborin G, Estey C, Moffat C, Crawford S, Saliba S, Jardine K, et al: Sirt1 regulates energy metabolism and response to caloric restriction in mice. *PLoS One* 2008;3:e1759.
- 68 Kume S, Uzu T, Horiike K, Chin-Kanasaki M, Isshiki K, Araki S, Sugimoto T, Haneda M, Kashiwagi A, Koya D: Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. *J Clin Invest* 2010;120:1043–1055.
- 69 Maeda S, Koya D, Araki S, Babazono T, Umezono T, Toyoda M, Kawai K, Imanishi M, Uzu T, Suzuki D, et al: Association between single nucleotide polymorphisms within genes encoding sirtuin families and diabetic nephropathy in Japanese subjects with type 2 diabetes. *Clin Exp Nephrol* 2011;15:381–390.
- 70 Shimoyama Y, Mitsuda Y, Tsuruta Y, Suzuki K, Hamajima N, Niwa T: *SIRTUIN 1* gene polymorphisms are associated with cholesterol metabolism and coronary artery calcification in Japanese hemodialysis patients. *J Ren Nutr* 2012;22:114–119.
- 71 Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D: Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* 2004;430:686–689.
- 72 Timmers S, Konings E, Bilet L, Houtkooper RH, van de Weijer T, Goossens GH, Hoeks J, van der Krieken S, Ryu D, Kersten S, et al: Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* 2011;14:612–622.
- 73 Gledhill JR, Montgomery MG, Leslie AG, Walker JE: Mechanism of inhibition of bovine F1-ATPase by resveratrol and related polyphenols. *Proc Natl Acad Sci USA* 2007;104:13632–13637.
- 74 Hubbard BP, Gomes AP, Dai H, Li J, Case AW, Considine T, Riera TV, Lee JE, E SY, Lamming DW, et al: Evidence for a common mechanism of SIRT1 regulation by allosteric activators. *Science* 2013;339:1216–1219.
- 75 Wong RH, Howe PR, Buckley JD, Coates AM, Kunz I, Berry NM: Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure. *Nutr Metab Cardiovasc Dis* 2011;21:851–856.
- 76 Crandall JP, Oram V, Trandafirescu G, Reid M, Kishore P, Hawkins M, Cohen HW, Barzilai N: Pilot study of resveratrol in older adults with impaired glucose tolerance. *J Gerontol A Biol Sci Med Sci* 2012;67:1307–1312.