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Nrf2 signalling promotes ex vivo tubular epithelial cell survival and regeneration via murine double minute (MDM)-2

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ABSTRACT

Background. Tubular repair upon injury involves regeneration from either surviving tubular epithelial cells or from their surviving local progenitor cells; hence, compound screening with cell lines may be inadequate. Here, we demonstrate that the renal cell isolation procedure and subsequent outgrowth of tubular cells can mimic the renal injury phase and tubular cell regeneration from whichever surviving renal cells.

Methods. We set up assays to systematically screen and identify mediators of tubular survival and repair.

Results. Forty-eight hours after plating total kidney isolates from C57BL/6 mice, 69% of cells survived when prepared from 2-week-old pups, but only 4% of cells from 8-week-old mice, respectively. This poor survival was not modulated by co-incubation with any of 24 cytokines and growth factors, except for the Nrf2 agonist sulforaphane. In addition, only sulforaphane enhanced the regenerative outgrowth of tubular epithelial cells from the mixed population. Furthermore, sulforaphane enhanced wound closure upon scratching tubular epithelial cell monolayers in a dose-dependent manner. This process was associated with the induction of the tested Nrf2 target genes HO-1, NQO1 and murine-double minute 2 (MDM2). MDM2 blockade with nutlin-3a completely blocked the protective effects of sulforaphane on renal cell survival, outgrowth and wound closure.

Conclusions. Together, renal cell isolation is a model of acute kidney injury (AKI). Primary tubular epithelial cell outgrowth represents a model of tubular regeneration. Nrf2 activation can enhance renal cell survival and tubular repair by inducing the cell cycle regulator MDM2.

INTRODUCTION

Acute kidney injury (AKI) mostly results from acute tubular necrosis (ATN) during ischaemic, septic and/or toxic injuries. AKI involves a series of danger response programmes that try to control the insult and to regain tissue homeostasis, including the induction of renal inflammation and subsequent renal repair [1–3]. Recovery from AKI largely depends on the regenerative potential of the surviving renal parenchymal cells which, as an ongoing controversy, may be differentiated/dedifferentiated epithelial cells or their local progenitor cells [4, 5]. Currently, no treatment is approved for the prevention of ATN or for the acceleration of renal repair upon ATN; hence, research efforts are needed, which allow systematic testing of compound libraries for their potential to either prevent renal cell death or accelerate renal repair. Cell lines are often used as a first preclinical assay system, but these do not include all the different cell populations that may be needed to repair differentiated epithelia from local progenitor cells. We, therefore, followed another strategy and set up a multistep in vitro assay system that uses the isolation procedure of primary tubular epithelial cells as a model of ischaemic tubular cell damage and studies the regenerative outgrowth from whichever cell that has the capacity to regenerate tubular epithelial cells. Using a set of different assays, we dissected stress resistance or capacity to survive oxidative and mechanical stress, regenerative outgrowth, migration and finally monolayer formation which are all needed to repair a functional monolayer of tubular epithelial cells inside the tubular compartment. As a proof-of-concept study we performed a systematic assessment of cytokines, chemokines and growth factors which have been implicated in the processes of tubular injury and repair.
We also studied sulforaphane, an agonist of the NF-E2-related factor (Nrf)-2. Nrf2 is a transcription factor that serves as a bottle neck as it translates the multiple triggers of oxidative stress into a coordinated transcription of hundreds of genes with anti-oxidative, cell protective and mitogenic effects [6, 7]. Nrf2 agonists such as sulforaphane or bardoxolone have already been shown to protect rodent kidneys from toxic and posts ischemic AKI (which was associated with induction of Nrf2-dependent genes [8, 9]) or even to improve the glomerular filtration rate in patients with advanced type 2 diabetic nephropathy [10].

We hypothesized that one or more of the tested compounds would increase the capacity of renal cells to survive oxidative stress ex-vivo and to accelerate the repair of a tubular epithelial cell monolayer from the surviving cells. In addition, we expected that our assay system reliably predicts the functional contribution of specific molecular targets or mediators for renal repair.

**MATERIALS AND METHODS**

**Renal cell culture**

Renal cell suspensions were prepared as previously described [11]. In brief, 2- or 8-week-old C57BL/6 mice were used for renal cell extraction. Kidneys were extracted, decapsulated, mashed into small pieces with sterile instruments and digested in 1 mg/ml collagenase A (Roche Diagnostics, Mannheim, Germany) for 30 min at 37°C. The material was pushed through a sieve of 70 μm pore diameter (BD, Franklin Lakes, NJ), washed and diluted in 2 ml of phosphate-buffered saline (PBS). Separation of the tubular segments was achieved through Percoll (31%) centrifugation at 3000 rpm/10 min/4°C. The pellet of tubular segments formed at the bottom of the tube was collected and washed twice with PBS at 1500 rpm/5 min/4°C. The renal cell isolates were cultured under sterile conditions at 37°C and 5% CO₂ in conditioned medium consisting of DMEM w/glucose (Gibco/Life Technologies, Grand Island, NY), 10% fetal bovine serum (Biochrom, Berlin, Germany), 1% penicillin/streptomycin (PAA Laboratories, Pasching, Austria), Hank’s Balanced Salt Solution (Sigma Aldrich, Steinheim, Germany), HEPES (Gibco/Life Technologies), EGF, T3, hydrocortisone, PGE-1 and insulin transferrin sodium selenite supplement (Roche Diagnostics). For stimulation experiments, recombinant murine cytokines (Immunotools, Friesoythe, Germany), sulforaphane (Biomol, Hamburg, Germany) or nutlin-3a (Alexis Biotech Ltd, London, UK) were added to the cell culture medium at different concentrations.

**Characterization of murine primary tubular epithelial cells**

The freshly isolated tubular segments were clearly identified as such by morphology on the phase-contrast microscope, as shown in Supplementary material, Figure S1A. After 2 days of culture, we observed outgrowth from successfully attached tubular segments, as shown in Supplementary material, Figure S1B. All other cells and particles were floating in the supernatant and represented dead cells which we proved by repetitive plating into new wells where the particles failed to subsequently attach and display outgrowth. To characterize cells of the outgrowing colonies and of the subsequent forming monolayers, we used immunostaining of acetaldehyde fixed cells on chamber slides (Nunc, NY) with primary antibodies for epithelial markers E-cadherin (mouse anti-mouse E-cadherin, BD Biosciences, Franklin Lakes), and cytokeratin-7 (RCK105, mouse anti-mouse cytokeratin-7, Abcam, Cambridge, UK). Stainings were evaluated using a standard fluorescence microscope or a LSM 510 confocal microscope and LSM software (Carl Zeiss AG).

Both of the cells of early colonies and of monolayers expressed the epithelial cell adhesion protein E-cadherin and the cytoskeleton protein cytokeratin 7 (Figure 1A). The purity of our tubular cell culture was assessed by staining for vimentin (D21H3, rabbit anti-mouse vimentin, Cell Signaling Technology, Danvers, MA), a marker of mesenchymal cells within the renal cell isolates. Only very few single vimentin + cells were spotted on Day 2 of culture, whereas some of the attached epithelial cells weakly co-expressed vimentin (Figure 1B), probably representing progeny of the scattered vimentin + intrarenal progenitor cells that derive from the metanephric mesenchyme and remain in an immature state in the adult kidney [4, 5]. During their proliferation and differentiation towards mature tubular epithelial cells, they slowly lose these mesenchymal markers. Due to the fast growth of these cells, the purity of epithelial cells reached nearly 100% at Day 5 of culture (Figure 1B).

**Flow cytometry analysis using the ALDEFLUOR assay kit**

The ALDEFLUOR® assay kit is used to mark all cells expressing the enzyme aldehyde dehydrogenase (ALDH) with a green fluorescent, which can then in turn be detected using a flow cytometer (FACS Calibur, BD). The preparation of samples and measurements were performed according to the manufacturer’s instructions (Stemcell Technologies, Grenoble, France).

**Quantification of the level of oxidative stress using the TBARS assay kit**

This kit serves as a measurement tool for intracellular concentration of malondialdehyde (MDA), which occurs as a side product during reagent oxygen species-induced formation of highly unstable lipid hydroperoxides. The kit was used according to the manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI).

**Assessment of cell survival**

The plates used in our regeneration assay were marked at the bottom in the patterns described in Supplementary material, Figure S2A. Freshly isolated tubular cells from two kidneys were separated into 24 equally large groups and distributed onto a 24-well cell culture plate containing 1 ml of culture medium per well. Treatment groups had a size of four wells per group, leading to a number of samples 8 (two pictures per well), considering the number of photographs. The
assessment of survival was performed 48 h after isolation, a time point that has been identified in a set of preliminary experiments as cells are unlikely to attach to the culture plate afterwards. Two images per well were taken at ×5 magnification using the digital phase-contrast microscope. The marks at the bottom of the plate ensured to always pick the same spot in each well. Supplementary material, Figure S3A shows two sample images from the survival assessment process. With our digital image analysis software, we counted both the number of tubular segments and colonies per picture, respectively. The survival score is the ratio of colonies per tubular fragments.

**Assessment of regenerative outgrowth**

The assessment of regeneration was performed in the same cell culture plates as the assessment of survival, in the sense of a continuous assay. Obviously, an additional marking on the bottom side of the plates was therefore not necessary. As before, treatment groups had a size of four wells per group, but with four photographs taken per time point, the number of samples was \( n = 16 \). Both single and multiple time point read-outs were possible with this kind of assay. Here, we show data from a single read-out 5 days after isolation. Four images per well were shot, each representing one of the squares on the bottom side. Supplementary material, Figure S3B, shows two sample images from the regeneration assessment process. Here again, all the acquired digital images were analysed with our digital image analysis software (Photoshop Extended CS5, Adobe Software). The size of the cell-covered area was put in proportion to the total image area size. The obtained quotient expresses regenerative outgrowth.

**Wound healing assay**

Scratch-induced wound healing assays were performed as previously described [12]. Prior to the assay, the plates were prepared as shown in Supplementary material, Figure S2B. Three hundred and fifty thousand cells per well were seeded into a 12-well cell culture plate. Upon the epithelial cells reaching confluency, the cells were starved in medium containing 2% fetal calf serum for 24 h. The cells were stimulated for 4 h prior to scratching. Scratching was performed manually with a pipette tip under standardized conditions. Two scratches per well, each of which were analysed on two different spots. Pictures of the wound were taken at 0 h and 24 h on a digital phase-contrast microscope, and then analysed using our digital image analysis software. Two observers

![FIGURE 1: Expression of tubular epithelial markers. Confocal and standard immunofluorescence microscopy of primary murine tubular epithelial cells in culture (chamber slides). (A) Expression of the cell adhesion protein E-cadherin and the cytoskeleton protein cytokeratin 7. Confocal microscopy. (B) Staining for the mesenchymal marker vimentin in young tubular cell colonies and in epithelial monolayers in culture. Standard fluorescence microscopy at ×20 magnification for Day 2 and ×10 magnification for Day 5.](image-url)
independently performed digital image analysis in order to minimize the possibility of cognitive bias. Change in wound sizes between two time points expressed the extent of healing in our experiment as shown in Supplementary material, Figure S3C.

**RNA preparation and real-time quantitative RT–PCR**

Reverse transcription and real-time RT–PCR from total primary tubular cell RNA was performed as described [13]. An SYBR Green dye detection system was used for quantitative real-time PCR on Light Cycler 480 (Roche, Mannheim, Germany). Gene-specific primers (300 nM, Metabion, Martinsried, Germany) were used as listed in Table 1. Controls consisting of ddH2O were negative for target and housekeeper genes.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism 5 Software (GraphPad Software). Data from vehicle or sulforaphane or other cytokine-treated cells were compared with analysis of variance followed by the Student–Newman–Keuls test. A T-test was used for direct comparisons between two groups. Data were expressed as mean ± standard error of the mean (SEM). A P value <0.05 was considered statistically significant.

**RESULTS**

**The isolation procedure itself represents an assay of tubular epithelial cell injury**

Kidney explantation, mechanical dissection and sieving expose the renal cells to mechanical and ischaemic, i.e. oxidative stress. Despite collagenase digestion this isolation method did not result in proper single cell suspensions but rather tubular fragments (Supplementary material, Figure S1A), of which only few were attached to the culture plate within 48 h. The attached fragments subsequently formed colonies of cellular outgrowth (Supplementary material, Figure S1B). The outgrowing cells were mostly tubular epithelial cells that rapidly overgrew all other cells, as evidenced by positive staining for E-cadherin and cytokeratin 7 and negative staining for vimentin (Figure 1). The non-adherent fragments did not attach when repetitively plated into new plates. Therefore, these fragments most likely consisted of renal cells that had not survived the isolation procedure. This is most likely to be accounted for by the significant increase of oxidative stress during the isolation procedure (Figure 2A). The rather small absolute change in MDA concentration in our assay eventually does not reflect its biological impact on the cells, whose stress fighting reserves are fully consumed. The surplus of stress due to mechanical stimulation then leads to cell death. Interestingly, MDA as a side product of ROS-triggered reactions itself contributes to the extent of the damage to the cell, acting as a toxic compound and leading to the formation of advanced lipoxidation end-products [14]. This underlines that the level of MDA does not have to be altered much to cause fatal consequences.

We wanted to know if also tubular progenitors would be included in the assay. We performed an experiment identifying cells that expressed the enzyme ALDH in an increased amount, which serves as a marker for stem cells or stem cell-like cells [5]. We could detect a small number of ALDHHigh cells in our heterogeneous tubular cell population using the ALDEFLUOR® fluorometric assay kit (Figure 2B). We conclude that our assay in fact not only depicts proliferation of surviving tubular cells upon injury, but also includes real regeneration from a small stem cell-like cell population. ALDHHigh progenitor cells not only permit a faster proliferation but also stress resistance as ALDH activity decreases the level of the toxic metabolite MDA.

<table>
<thead>
<tr>
<th>Target</th>
<th>Primer sequence</th>
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<tr>
<td>MDM2</td>
<td>Forward primer</td>
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<tr>
<td></td>
<td>5’-CCCCGAGTTTCTCTGTGAAGG-3’</td>
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<td></td>
<td>Reverse primer</td>
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<td></td>
<td>5’-TCCTTCAGATCACTCCACC-3’</td>
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<tr>
<td>Nrf-2</td>
<td>Forward primer</td>
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<tr>
<td></td>
<td>5’-CTCAGCATGATGGACCTTGA-3’</td>
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<td></td>
<td>5’-TCTATGTCCTGCCCTCCAAAGG-3’</td>
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<tr>
<td>NQO1</td>
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<td>5’-TTAGGGGTCGTCCTGGACA-3’</td>
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<td>5’-GTCCTTCGTCCTGAAATTGGCAG-3’</td>
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<td>HO-1</td>
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<td>5’-GAGCCTGAAATCGACAGA-3’</td>
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<td></td>
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The percentage of adherent tubular fragments after 48 h to the number of fragments that had been plated directly after the isolation procedure was 69% in 2-week-old mice and 4% in 8-week-old mice (Figure 2C). We conclude that from adult mouse kidney only few stress-resistant renal cells survive the isolation procedure, while younger mice seem to have more cells that can sustain the oxidative stress of the isolation procedure.

**Nrf2 stabilization with sulforaphane increases survival of renal cells upon isolation**

To investigate how to modulate renal cell survival during injury, we launched a screening experiment with certain candidate compounds. For example, sulforaphane is a compound with antioxidant effects because it stabilizes Nrf2 by blocking its ubiquitination [9, 15]. We hypothesized that the anti-oxidative effects of Nrf2 stabilization would have beneficial effects on renal cell survival upon injury. Freshly isolated renal fragments were immediately incubated with sulforaphane or vehicle at different concentrations. In addition, we exposed renal fragments to a set of recombinant murine cytokines and chemokines. Sulforaphane significantly increased the number of adherent tubular fragments in a dose-dependent manner up to 5.5-fold in comparison to the vehicle group (Figure 3A). It is of note that none of the other compounds showed a significant increase of renal cell survival (Figure 3A).

**Nrf2 stabilization with sulforaphane promotes regenerative outgrowth of tubular epithelial cells**

In the next phase of our screening approach, we monitored the regenerative outgrowth of the renal cells from...
the adherent tubular fragments and their capacity to form an epithelial monolayer in vitro. After several rounds of pilot experiments, we standardized the procedure by quantifying the degree of confluency after 5 days of culture as described in methods. Consistent with its effect on cell survival, sulforaphane also significantly accelerated the regenerative outgrowth by over 2.5-fold compared with the vehicle group (Figure 3B). The regenerative outgrowth eventually resulted in a tubular epithelial monolayer of high purity. Together, Nrf2 stabilization with sulforaphane increases the regenerative tubular cell outgrowth from renal cell isolates.

**Nrf2 stabilization with sulforaphane accelerates tubular epithelial wound healing**

Even though renal epithelial cells grow in convoluted and straight tube-like structures, i.e. the cortical or medullary tubules, they conceptually form a single monolayer epithelium. Repair of monolayer injury may include different signals to repair the wound than simple cell proliferation in a culture dish, like, e.g. activation of growth-arrested cells, proliferation and migration to close a wound [16]. To mimic this process in vitro, we performed scratch-induced injury of tubular epithelial cell monolayers in culture. Figure 4 shows wound closure at 24 h after scratching. Sulforaphane...
significantly enhanced wound closure speed in a dose-dependent manner in comparison to the control group. We conclude that sulforaphane not only increases tubular cell survival but also monolayer repair upon (mechanical) injury.

**Nrf2 activation with sulforaphane upregulates anti-oxidant-related genes**

Sulforaphane acts as an Nrf2 agonist by releasing Nrf2 from its binding molecule KEAP1 [7, 15]. Upon activation, Nrf2 translocates to the nucleus where it acts as a transcription factor driving the expression of phase-2 ROS-fighting genes (reactive oxygen species). Therefore, we questioned whether such genes were also upregulated in our experiments. We isolated whole RNA from the cells after successful completion of the Scratch assay. Nrf2 itself as well as the NAD(P)H dehydrogenase (quinone) 1 (NQO1) and the haeme oxygenase 1 (HO-1) were significantly upregulated in a dose-dependent manner after sulforaphane stimulation (Figure 5A). Thus, the Nrf2 agonist sulforaphane induces anti-oxidative factors in tubular epithelial cells which may contribute to its effects on cell survival and regeneration.

**The pro-survival and pro-regeneratory effects of sulforaphane involve MDM2**

It has been described that Nrf-2 overexpression also leads to an upregulation of the cell cycle regulator murine-double minute 2 (MDM2) [17], an E3 ubiquitin ligase that promotes cell survival and growth by inactivating p53 [18, 19]. We recently demonstrated that the mitogenic effects of MDM2 promote tubular regeneration after post-ischaemic tubular necrosis [20]. Therefore, we speculated that MDM2 might contribute to the tubular regeneration upon injury in vitro as well as in vivo. First, we quantified MDM2 mRNA induction in tubular epithelial cells upon sulforaphane exposure. Here again, we collected whole RNA from the cells after successful completion of the Scratch assay. MDM2 mRNA was significantly upregulated in a dose-dependent manner (Figure 5B). In order to study the functional contribution of MDM2 in sulforaphane-induced acceleration of wound healing, we used the MDM2 inhibitor nutlin-3a [21–23]. We then included nutlin-3a in the first step of our assay. Nutlin-3a-treatment led to a significant drop in cell survival upon sulforaphane treatment when compared with sulforaphane alone (Figure 6A). To show that the MDM2 upregulation upon sulforaphane stimulation had a functional impact on proliferation, we co-stimulated the cells with sulforaphane and nutlin-3a in Step 2 of the regeneration part of our screening assay. Nutlin-3a plus sulforaphane also significantly decreased regenerative outgrowth in comparison to the sulforaphane alone (Figure 6B). Finally, we stimulated the cells with either sulforaphane, nutlin-3a or sulforaphane w/nutlin-3a in the scratch wounding assay. Nutlin-3a decelerated wound closure speed in comparison to the control group (Figure 6C). Co-stimulation with sulforaphane and nutlin-3a could equalize the negative effect of nutlin-3a.

**FIGURE 4:** Wound healing assay of tubular cell monolayers. Primary murine tubular epithelial cells from adult C57BL6 wild-type mice were grown to confluency in 12-well culture plates. Scratching was performed after starving the cells in FBS-reduced medium for 24 h. Cells were stimulated either with DMSO or DMSO w/sulforaphane in different dosages. Data are expressed as mean fold change ± SEM in wound closure speed compared with the control group. N = 16. *P < 0.05, **P < 0.01, ***P < 0.001.

**FIGURE 5:** mRNA Expression of Phase-2 genes and MDM2. Total RNA was collected from primary tubular cells after the scratch assay experiment was finished. Nrf-2, NQO-1, HO-1 (A), and MDM2 (B) mRNA was then quantified by qRT–PCR. Data are expressed as mean ratio ± SEM to 18S mRNA expression, a housekeeping gene. Darker colouring represents increasing dosage of sulforaphane, as indicated below the x-axis. n = 16. P < 0.01.

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These data suggest that MDM2 protects from tubular cell death during injury and promotes tubular repair. The Nrf2 agonist sulforaphane induces MDM2 which contributes to the beneficial effects of sulforaphane on tubular cell survival and tubular regeneration (Figure 7).

**DISCUSSION**

The mechanisms that promote or limit renal regeneration upon kidney injury are currently under intense investigation, but it remains a matter of fact that up to now not a single drug has been approved to specifically stimulate renal repair. Traditionally, the concept of repair is limited to cell proliferation and often mimicked by proliferation assays with cell lines in culture. As of now, evidence exists for tubular repair originating from differentiated or dedifferentiated tubular epithelial cells [24, 25] or intrarenal tubular cell progenitors [4, 5], supported by pro-regenerative mediators released from intrarenal macrophages and dendritic cells [26–28]. Hence, we decided to perform screening studies only with renal cell suspensions to assess whether the renal cell isolation procedure by itself represents a model of AKI and whether it can help us to identify extracellular mediators of tubular repair.

Consistent with this view, we found that only very few clusters of cells survive the isolation procedure when isolated...
from adult mice. However, we found that renal cell isolation from 2-week-old pups significantly increased the yield of surviving renal cells which documents increased resistance to mechanical and oxidative stress of renal cells and might relate to a higher number of stress-resistant tubular cells at younger age. Nevertheless, solving the ongoing discussion on the cellular source of tubular repair was not the focus of the present study.

Our screening approach included a number of cytokines, chemokines and growth factors many of them known to be involved in renal ischaemia–reperfusion injury. None of them directly affected renal cell survival in a significant manner, except for sulforaphane, which also enhanced the regenerative outgrowth of tubular epithelial cells and wound closure of tubular cell monolayers. Sulforaphane is a well-known agonist for Nrf2, a transcription factor and master regulator of several hundred genes that confer anti-oxidative effects [7, 15]. Nrf2 agonists protect rodent kidneys from toxic and post-ischaemic AKI by inducing anti-oxidative genes [8, 9, 29]. We specifically focused on the Nrf2 target gene MDM2 because we recently identified MDM2 to be an important mediator of tubular cell regeneration upon post-ischaemic AKI [20]. MDM2 is an E3 ligase that inhibits the transcription factor p53, the master regulator of cell cycle arrest and apoptosis. MDM2 overexpression suppresses p53 and thereby promotes cell growth, e.g. in malignant cells [19, 22], in lymphocytes during autoimmune lymphoproliferative syndromes [30], and obviously in tissue regeneration upon sterile injury [20]. Our studies during low here first demonstrate that Nrf2 induces MDM2 in renal cells which then contributes to renal cell survival during kidney injury, directly stimulates regenerative proliferation from surviving tubular (progenitor) cells and thereby promotes healing of tubular cell monolayers. Therefore, even though pharmacological MDM2 inhibition with nutlins may be a valid strategy to reduce tumour growth or systemic autoimmunity [20, 22, 23], it may rather be desirable to enhance MDM2 expression during tissue injury and wound healing. Our studies here now suggest that an agonist of Nrf2 may induce MDM2 together with many other anti-oxidative genes which could be an even more favourable strategy to improve cell survival during cell repair after injury.

In summary, preparing renal cell suspensions represents a model of AKI during which most of the cells die. Regenerative outgrowth evolves from a small number of cells that survive the acute injury, which can be used as a model of (tubular) cell regeneration. The influence of infiltrating leukocytes and fibroblasts is not included in this experimental setting and therefore, has to be the subject of expanded studies. Scratch assays complement the assay by testing the capacity of wound healing of injured tubular epithelial cell monolayers. Systematic screening identified Nrf2 as a novel therapeutic target to increase renal cell survival and regeneration. Nrf2 mediates its pro-survival and pro-regenerative effects via the MDM2-mediated suppression of cell cycle regulator p53. Our data suggest that Nrf2 agonists may protect renal cells during kidney injury and may accelerate renal repair, which could have wide clinical implications in the field of nephrology.

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**SUPPLEMENTARY DATA**

Supplementary data are available online at http://ndt.oxfordjournals.org.

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**CONFLICT OF INTEREST STATEMENT**

None declared.

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