

AMD1-Mediated Polyamine Metabolism Governs Tubular Repair Fate by Restraining Senescence after Kidney Injury

Baiwei Mao

Shanghai First People's Hospital

Zhihuang Zheng

Shanghai First People's Hospital

Wenxin Fu

Shanghai First People's Hospital

Guozhe Cheng

Shanghai First People's Hospital

Lijun Wang

Shanghai First People's Hospital

Jinfang Bao

Shanghai First People's Hospital

Xiaohua Liu

Department of Nephrology, Ningde Municipal Hospital of Ningde Normal University

Hongbin Zhan

Department of Nephrology, Ningde Municipal Hospital of Ningde Normal University

Miao Pan

Department of Nephrology, Ningde Municipal Hospital of Ningde Normal University

Jun Liu

`liujun-sgh@sjtu.edu.cn`

Shanghai First People's Hospital

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Abstract

Failure of adaptive repair after acute kidney injury (AKI) drives the transition to chronic kidney disease (CKD), yet the metabolic checkpoints governing tubular fate remain incompletely defined. Here, we investigated whether the polyamine biosynthetic enzyme S-adenosylmethionine decarboxylase 1 (SAMD1/AMD1) regulates tubular senescence and repair outcomes after kidney injury and elucidated the underlying mechanism. Key metabolic pathways and candidate enzymes associated with AKI progression were identified by bioinformatics analyses. AMD1 dynamics were examined in a mouse ischemia–reperfusion injury (IRI) model by immunofluorescence. Proximal tubule–specific *Amd1* conditional knockout mice (*Amd1*^{cko}) were used to assess renal injury, cell-cycle status, senescence, and remodeling, and exogenous spermidine (Spd) was administered for rescue. DNA damage signaling and p53/p21 activation were evaluated by immunostaining/Western blotting and EdU incorporation assays. AMD1 was predominantly expressed in proximal tubules, showed compensatory induction early after IRI, and was markedly downregulated during the late phase, correlating inversely with fibrosis. Compared with wild-type controls, *Amd1*^{cko} mice exhibited aggravated tubular injury, a two-fold increase in SA- β -gal–positive areas ($\approx 45\%$ vs. $\approx 20\%$), elevated p21, and reduced Ki67+ proliferation. Spd supplementation improved renal function (BUN protection rate 71.04%), reduced fibrosis by 75.3%, and decreased senescent regions by $\sim 74\%$. Mechanistically, AMD1 loss increased γ H2AX-marked DNA damage and activated the p53/p21 checkpoint, whereas Spd attenuated this response and restored DNA synthesis capacity. Collectively, tubular AMD1 acts as a metabolic checkpoint that preserves polyamine homeostasis to restrain p53/p21-dependent senescence and promote adaptive repair after AKI, and spermidine supplementation represents a potential strategy to mitigate maladaptive AKI-to-CKD progression.

1. Introduction

Acute kidney injury (AKI) is increasingly recognized as a pivotal turning point rather than a fully reversible episode. This is especially true in older patients, in whom AKI frequently progresses to chronic kidney disease (CKD) due to failure of adaptive repair^{1–3}. A central determinant of this AKI-to-CKD transition is the fate of injured tubular epithelial cells: successful repair requires cell-cycle re-entry and regeneration, whereas maladaptive repair is characterized by sustained cell-cycle arrest and cellular senescence. Senescent tubules are not inert; through the persistent production of a senescence-associated secretory phenotype (SASP), they amplify chronic inflammation, remodel the interstitium, and accelerate tubulointerstitial fibrosis⁴. Thus, identifying upstream, druggable mechanisms that govern the “repair-versus-senescence” decision has major clinical relevance for preventing CKD after AKI in aging populations.

Metabolic reprogramming has emerged as a key regulator of tubular fate. While prior studies have largely focused on lipid dysregulation, mitochondrial dysfunction and glycolytic rewiring^{5,6}, the contribution of amino acid–derived metabolites remains underexplored. Polyamines are cationic metabolites essential for chromatin organization, DNA repair, and cell-cycle progression, and their tissue

levels decline markedly with aging^{7–9}. Despite growing links between polyamine metabolism and aging phenotypes¹⁰, how polyamine homeostasis shapes tubular repair trajectories during AKI-to-CKD progression is still poorly defined.

S-adenosylmethionine decarboxylase 1 (AMD1) is a rate-limiting enzyme in polyamine biosynthesis and a metabolic node connecting polyamine production with one-carbon metabolism^{7,11}. Previous work has emphasized AMD1-mediated perturbation of methyl-donor balance (SAM/dcSAM) and epigenetic instability; however, this methylation-centered view may overlook AMD1's "gatekeeper" role in supplying higher bioactive polyamines, such as spermidine and spermine, that directly support genome integrity and proliferative competence¹¹.

Here, we integrate public transcriptomic mining with in vivo and in vitro validation to identify AMD1 as a proximal tubule–enriched enzyme that becomes markedly downregulated during late-phase post-ischemic injury. Using proximal tubule–specific *Amd1* conditional knockout mice, we show that tubular AMD1 loss exacerbates renal dysfunction and fibrotic remodeling by promoting DNA damage accumulation and activating the p53/p21 checkpoint, thereby enforcing tubular senescence and SASP programs. Importantly, exogenous spermidine supplementation suppresses p53/p21 activation, restores DNA synthesis and proliferation, and markedly improves functional and structural repair. Together, these findings establish AMD1 as a metabolic checkpoint controlling post-injury tubular fate and highlight polyamine restoration as a potential strategy to mitigate maladaptive AKI-to-CKD progression.

2. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

2.1. Downregulation of the Polyamine Enzyme AMD1 Is Closely Associated with Impaired Repair and Activation of Senescence Programs after IRI

To explore metabolic determinants underlying maladaptive repair during the AKI-to-CKD transition, we interrogated a public bulk RNA-seq dataset of mouse renal ischemia–reperfusion injury (IRI) (GSE165876). Principal component analysis (PCA) showed a clear separation between Sham and IRI kidneys across the post-injury time course, indicating robust transcriptional reprogramming during injury and repair (Figure 1A). We then performed pathway-level interrogation using KEGG ssGSEA and found that amino acid/nitrogen metabolism programs containing a polyamine-related module exhibited a biphasic pattern, with early compensatory activation after IRI followed by marked suppression at the late stage (3 weeks) (Figure 1B).

Focusing on the polyamine module, heatmap profiling of core polyamine genes revealed that AMD1 displayed the most prominent downregulation in late-phase IRI, suggesting that AMD1 may represent a key metabolic bottleneck in this context (Figure 1C). To resolve the cellular origin of AMD1 within the kidney, we examined single-cell RNA-seq expression patterns from the Susztak Lab Kidney Biobank—

Mouse Kidney IRI Atlas[12] and observed that Amd1 expression was enriched in proximal tubular epithelial cells, with comparatively lower expression in other renal cell populations (Figure 1D).

Next, we asked whether polyamine metabolism dynamics track with established injury/repair states after IRI. Module-based analyses demonstrated that the polyamine score declined in parallel with increased senescence- and fibrosis-associated signatures, consistent with an inverse relationship between polyamine metabolic capacity and maladaptive repair features (Figure 1E). In line with this, bulk expression analysis confirmed that Amd1 mRNA levels were significantly reduced in IRI kidneys compared with Sham controls, particularly at later time points (Figure 1F). Correlation analyses further supported the clinical-pathological relevance of this axis: Amd1 expression negatively correlated with the fibrosis module (Figure 1G), while showing a positive association with the repair index (Figure 1H).

Collectively, these data identify tubular AMD1 downregulation as a characteristic late-stage event after renal IRI and link reduced AMD1/polyamine metabolic activity to fibrotic remodeling and senescence-associated maladaptive repair, implicating AMD1 insufficiency as a potential metabolic trigger for senescence/SASP programs during AKI-to-CKD progression.

2.2. In Situ Validation Reveals a Biphasic Induction of Tubular AMD1 after IRI and Its Inverse Association with Fibrotic Remodeling

To validate the bioinformatics-derived AMD1 dynamics at the tissue level, we performed multiplex immunofluorescence staining in a mouse renal IRI model to map the spatiotemporal expression of AMD1 within proximal tubules. Under basal conditions (Sham), AMD1 exhibited low-level constitutive staining in AQP1+ proximal tubules (Figure 2A). Strikingly, during the acute injury phase (24 h and 72 h post-IRI), AMD1 fluorescence intensity was markedly increased in proximal tubules, indicating an early injury-responsive induction (Figure 2A). Co-staining with the injury marker KIM-1 further demonstrated that AMD1 upregulation was preferentially enriched in KIM-1+ damaged tubules, suggesting that AMD1 is activated predominantly within injured epithelial compartments during early post-ischemic stress (Figure 2A, right). However, as the injury course progressed to day 14, AMD1 signal declined relative to the acute phase, revealing a late-stage “AMD1 insufficiency” pattern consistent with the transcriptomic suppression observed during maladaptive repair (Figure 2A).

We next asked whether AMD1 dynamics track with epithelial proliferative responses. Co-immunofluorescence for Ki67 showed that tubular proliferation peaked at 24 h, coinciding with the early rise in AMD1 expression, but declined substantially by day 14, when AMD1 levels had fallen (Figure 2B). This temporal coupling supports the notion that AMD1-associated metabolic capacity may be linked to the regenerative competence of proximal tubular cells during repair.

Finally, to connect late-phase AMD1 loss with tissue remodeling, we assessed interstitial activation/fibrosis using α -SMA staining. Compared with earlier time points, α -SMA+ area was prominently expanded at day 14 post-IRI, coinciding with reduced AMD1 intensity in proximal tubules (Figure 2C). Quantitative correlation analysis further confirmed that tubular AMD1 intensity was

significantly and inversely correlated with α -SMA+ area (Figure 2C), supporting a tight association between declining AMD1 in proximal tubules and the emergence of fibrotic remodeling.

Taken together, these in situ data establish that AMD1 undergoes a biphasic response after renal IRI—early induction in KIM-1+ injured proximal tubules followed by late-stage downregulation—and that reduced tubular AMD1 is coupled to diminished proliferative activity and enhanced fibrotic progression, implicating AMD1-linked polyamine metabolism as a key metabolic feature of maladaptive repair.

2.3. Tubular AMD1 Deficiency Drives Cell-Cycle Arrest and Senescence, Leading to Maladaptive Repair after AKI

To directly determine whether tubular AMD1 is functionally required for post-AKI repair, we subjected proximal tubule-specific *Amd1* knockout mice (*Amd1^{cko}*) and littermate WT controls to renal IRI and evaluated repair outcomes at the late phase. Histological assessment by H&E staining revealed that, compared with WT-AKI kidneys, *Amd1^{cko}*-AKI mice displayed more severe tubular damage, accompanied by a significantly higher tubular injury score (Figure 3A). Consistent with these findings, Masson's trichrome staining demonstrated that interstitial collagen deposition was markedly increased in *Amd1^{cko}* kidneys, with quantitative analysis confirming a significant elevation in relative fibrosis area compared with WT-AKI controls (Figure 3B).

Given that persistent senescence is a hallmark of maladaptive repair, we next examined senescence burden using SA- β -Gal staining. While senescence was induced after IRI in WT kidneys, *Amd1^{cko}* mice exhibited a striking exacerbation of SA- β -Gal positivity, indicating a substantial expansion of senescent tubular areas (Figure 3C).

We then asked whether this repair failure was associated with impaired proliferative regeneration and activation of cell-cycle arrest pathways. Multiplex immunofluorescence staining in AQP1+ proximal tubules showed that Ki67+ proliferating tubular cells increased in WT kidneys after IRI, reflecting a regenerative response; however, this proliferative response was significantly blunted in *Amd1^{cko}* kidneys, as evidenced by a marked reduction in the proportion of Ki67+ cells (Figure 3D). In parallel, immunostaining for the cyclin-dependent kinase inhibitor p21 revealed that p21 expression was robustly upregulated after IRI and further intensified in *Amd1^{cko}* kidneys, supporting enhanced cell-cycle arrest in the absence of AMD1 (Figure 3E).

Collectively, these results demonstrate that loss of tubular AMD1 shifts the post-IRI trajectory toward persistent p21-associated cell-cycle arrest and heightened senescence, thereby suppressing tubular proliferative repair and promoting fibrotic remodeling—features consistent with maladaptive repair during the AKI-to-CKD transition.

2.4. Exogenous Spermidine Supplementation Markedly Alleviates AMD1-Deficiency–Induced Renal Injury, Senescence, and Interstitial Fibrosis

To determine whether re-establishing polyamine homeostasis could rescue the maladaptive phenotype caused by tubular AMD1 loss, we administered exogenous spermidine (Spd) to *Amd1^{cko}* mice after IRI. Serum biochemistry analyses showed that, compared with the *Amd1^{cko}*-AKI group, Spd significantly reduced serum creatinine (Scr) and blood urea nitrogen (BUN) (Figure 4A,B). When expressed as protection rates, Spd conferred 71.04% protection for BUN and 50.97% for Scr (Figure 4C), indicating a robust improvement in renal function despite AMD1 deficiency.

Histopathological evaluation further supported these functional benefits. Masson's trichrome staining demonstrated that Spd treatment markedly decreased collagen deposition and interstitial fibrosis in *Amd1^{cko}* kidneys, reducing the fibrotic area from approximately 17% to 5% and yielding a 75.3% fibrosis protection rate (Figure 4D,E).

We next investigated whether Spd restored the imbalance between proliferation and senescence that characterizes maladaptive repair. SA- β -Gal staining revealed that Spd markedly reduced the senescent area in *Amd1^{cko}* kidneys, corresponding to an estimated 74% protection rate against senescence (Figure 4F,G). Consistently, immunofluorescence analyses showed that Spd suppressed the aberrantly activated "cell-cycle arrest-senescence" axis in injured proximal tubules: p21 positivity decreased from ~58% to ~18%, whereas the proportion of Ki67+ proliferating tubular cells increased from ~10% to ~36% (Figure 4H,I).

Collectively, these results demonstrate that exogenous spermidine effectively corrects AMD1-deficiency-induced polyamine depletion, restrains tubular senescence, re-enables cell-cycle re-entry, and thereby suppresses chronic fibrotic remodeling while promoting functional renal repair after AKI.

2.5. AMD1 Deficiency Activates the DNA Damage-p53/p21 Axis and Induces SASP Programs, Which Are Reversed by Spermidine

To elucidate the mechanism by which AMD1 loss drives tubular senescence, we examined the DNA damage response and the p53/p21 checkpoint pathway. Immunohistochemistry for γ H2AX revealed that, compared with WT kidneys, *Amd1^{cko}* kidneys displayed a significant increase in γ H2AX-positive areas, indicating enhanced DNA damage accumulation and genomic instability upon AMD1 deficiency (Figure 5A). Western blotting further confirmed that DNA damage was accompanied by activation of the p53/p21 axis, as evidenced by increased levels of phosphorylated p53 (p-p53) and p21 in *Amd1^{cko}* kidneys (Figure 5B). Importantly, Spd supplementation markedly reduced γ H2AX positivity and suppressed p-p53 and p21 induction (Figure 5A,B), suggesting that restoring polyamine availability mitigates DNA damage-driven checkpoint activation.

Because senescence is tightly linked to a pro-inflammatory secretory phenotype, we next assessed SASP-related transcripts. RT-qPCR analyses showed that *Amd1^{cko}* kidneys exhibited elevated expression of Il1b, Il6, Cdkn1a, and Cdkn2a, consistent with amplification of senescence-associated inflammation, whereas Spd treatment broadly attenuated these SASP components (Figure 5C).

Finally, we validated the impact of this metabolic axis on proliferative capacity in vitro using an HK-2 hypoxia/reoxygenation (H/R) model. AMD1 knockdown severely impaired DNA synthesis, reducing the EdU-positive fraction to approximately 6.5%, while Spd supplementation restored EdU incorporation to ~42.8% (Figure 5D).

Together, these findings indicate that AMD1 preserves tubular proliferative competence by maintaining genome integrity and restraining DNA damage–p53/p21 checkpoint activation, thereby preventing the establishment of a self-reinforcing “damage–senescence–inflammation” loop. Restoration of polyamine levels by spermidine interrupts this cascade and promotes adaptive repair following AKI.

3. Discussion

Failure of adaptive repair after acute kidney injury (AKI) is a major determinant of chronic kidney disease (CKD) progression, particularly in older populations². In this study, we identify S-adenosylmethionine decarboxylase 1 (AMD1) as a proximal tubule–enriched metabolic checkpoint that shapes the post-injury “repair-versus-senescence” fate decision[6],[13]. By integrating public transcriptomic analyses with in vivo and in vitro validation, we show that polyamine metabolism displays a biphasic response after ischemia–reperfusion injury (IRI), with early compensatory activation followed by late suppression coincident with maladaptive remodeling[14],[15]. AMD1 emerged as the most prominently downregulated enzyme in the late phase, and its tubular expression inversely correlated with fibrosis. Functionally, proximal tubule–specific Amd1 deletion exacerbated renal dysfunction and fibrotic remodeling while enforcing cell-cycle arrest and senescence[16], whereas restoring polyamine availability with spermidine robustly improved renal function and mitigated senescence and fibrosis. Collectively, our findings position AMD1-mediated polyamine homeostasis as an actionable metabolic vulnerability underlying the AKI-to-CKD transition.

A central advance of this work is the clarification of AMD1’s polyamine-centric function in kidney repair, which complements—and in the post-AKI setting may outweigh—its traditionally emphasized linkage to methyl metabolism[7],[11]. AMD1 has long been discussed through the lens of one-carbon metabolism because it consumes S-adenosylmethionine (SAM) and generates decarboxylated SAM (dcSAM), a metabolite that can inhibit DNA methyltransferases and thereby influence epigenetic landscapes[17], [18]. While such methyl-donor perturbations may contribute to aging-associated phenotypes, they do not readily explain the rapid shifts in tubular proliferation, checkpoint activation, and fibrotic remodeling observed during repair trajectories. Here, the ability of spermidine supplementation to broadly rescue renal function, suppress senescence, and attenuate fibrosis in AMD1-deficient kidneys argues that the dominant, therapeutically tractable consequence of AMD1 insufficiency during AKI-to-CKD progression is impaired production of higher bioactive polyamines. In this context, AMD1 should be viewed as a rate-limiting metabolic node that sustains polyamine pools required for genome stability and regenerative competence.

Mechanistically, our data support a model in which AMD1 insufficiency drives maladaptive repair by amplifying DNA damage signaling and enforcing the p53/p21 checkpoint, thereby locking injured tubules into a senescent, SASP-producing state[19]. Polyamines are essential cationic metabolites that stabilize chromatin, support DNA repair, and facilitate cell-cycle progression. In *Amd1^{cko}* kidneys, increased γH2AX accumulation, elevated p53 phosphorylation, and upregulated p21 collectively indicate intensified DNA damage burden and strengthened checkpoint activity[20–22]. This molecular program was accompanied by increased expression of senescence- and SASP-associated transcripts, consistent with a microenvironment that sustains chronic inflammation and promotes fibrotic remodeling[23]. Importantly, spermidine supplementation attenuated DNA damage markers, dampened p53/p21 activation, and restored DNA synthesis capacity, linking polyamine replenishment to reversal of the senescence barrier and re-entry into regenerative programs[9],[24]. Together, these findings provide a mechanistic bridge between metabolic insufficiency and a well-established cell-fate axis that determines repair outcome.

From a translational perspective, the magnitude of benefit achieved by polyamine restoration is notable. Spermidine improved renal function, reduced collagen deposition, decreased senescence burden, and increased tubular proliferation in AMD1-deficient kidneys²⁵. Beyond replenishing a limiting metabolite, spermidine may confer layered protection relevant to current molecular trends in kidney disease, including enhancement of autophagic clearance of damaged organelles, mitigation of oxidative stress, and modulation of inflammatory signaling[24],[26],[27]. These pleiotropic properties may be particularly advantageous in aged kidneys, where diminished metabolic flexibility and reduced stress-buffering capacity bias tubular cells toward checkpoint activation and senescence[6],[28]. Thus, targeting the AMD1–polyamine axis may represent a pragmatic strategy to interrupt the “damage–senescence–inflammation” loop that underlies maladaptive AKI-to-CKD progression.

Several limitations should be acknowledged. First, although our data support a polyamine-dependent mechanism, direct quantification of renal polyamine species (putrescine, spermidine, spermine) and metabolic flux across time points and tubular states would strengthen causal inference and clarify whether specific polyamines dominate the protective effect. Second, the downstream network likely extends beyond p53/p21; identifying additional nodes—such as ROS/mitochondrial stress responses, ATM/ATR–CHK signaling, NF-κB activation, or chromatin remodeling factors—will be important to fully map how polyamine depletion is translated into a stable senescence program[21],[29],[30]. Third, our conditional knockout model captures a genetic loss-of-function state; whether AMD1 downregulation in clinical AKI is driven by transcriptional repression, altered substrate availability, post-translational regulation, or shifts in tubular cell states (e.g., failed-repair subsets) remains to be determined[31],[32]. Fourth, although spermidine was effective in our setting, optimal dosing, timing, and delivery routes—and the relative efficacy of prevention versus reversal of established senescence—require systematic definition to improve translational relevance[27],[33]. Finally, because AKI-to-CKD risk is highest in the elderly, validation in naturally aged animals and in comorbidity models (e.g., diabetes, hypertension) will be essential to assess robustness and safety under clinically relevant conditions[28].

In conclusion, we identify tubular AMD1 downregulation as a key driver of maladaptive repair after AKI. By maintaining polyamine homeostasis, AMD1 preserves genome integrity and restrains p53/p21-dependent senescence, thereby limiting SASP amplification and fibrotic remodeling. Restoration of polyamine availability—most directly through spermidine supplementation—offers a promising metabolic strategy to promote adaptive tubular repair and mitigate AKI-to-CKD progression.

4. Materials and Methods

Animals and Reagents

8-week-old male C57BL/6J mice (23–25 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. and housed under specific pathogen-free (SPF) conditions at the Experimental Animal Center of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine. All procedures were approved by the institutional ethics committee (Approval No. 2021AW016) and were conducted in accordance with relevant guidelines for the humane use and care of laboratory animals.

Primary antibodies were obtained from the following sources: AMD1 and α -smooth muscle actin (α -SMA) (Abcam); β -actin (Cell Signaling Technology, CST); aquaporin-1 (AQP1), kidney injury molecule-1 (KIM-1), and Ki67 (Proteintech); and p21 (Santa Cruz Biotechnology). Blood urea nitrogen (BUN) and serum creatinine (Scr) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute. RT-qPCR reagents were obtained from Vazyme (Nanjing, China).

Experimental Design and Renal Ischemia–Reperfusion Injury Model

Mice were randomly assigned to four groups ($n = 5$ per group): Sham (control), AKI (IRI), $Amd1^{cko}$ -AKI, and $Amd1^{cko}$ + spermidine (Spd)-AKI. AKI was induced by bilateral renal pedicle clamping for 25 min followed by reperfusion. Body temperature was maintained at 37 °C throughout surgery. Sham-operated mice underwent identical procedures except for vascular clamping. Mice were euthanized on day 14 after surgery; serum was collected, and kidneys were harvested. Kidney tissues were transversely sectioned and either fixed in 4% paraformaldehyde or snap-frozen and stored at –80 °C for subsequent analyses.

In Situ Renal Delivery of AAV

Two weeks before IRI surgery, adeno-associated virus (AAV)-shAMD1 or AAV-control was delivered by bilateral renal pelvis microinjection (50 μ L per side) using a microsyringe. The needle was retained in place for 1 min to facilitate viral dispersion. Mice were then returned to standard housing conditions to allow stable transgene expression before AKI induction.

Spermidine Administration

Spermidine (Spd; Selleck Chemicals) was dissolved in sterile normal saline. From the day of IRI, mice in the Spd group received Spd by oral gavage at 10 mg/kg/day for 14 consecutive days. Control animals

received an equal volume of normal saline.

Histopathology and Tubular Injury/Fibrosis Scoring

Kidney sections were stained with hematoxylin and eosin (H&E) for histological assessment and Masson's trichrome staining for evaluation of fibrosis. For each sample, 20 random fields were captured, and tubular injury was scored based on the percentage of damaged tubules (injury area proportion). Fibrotic area was quantified from Masson-stained sections using ImageJ.

Serum Biochemistry

Peripheral blood was collected and centrifuged at 3000 rpm for 15 min to obtain serum. Scr and BUN levels were determined according to the manufacturer's instructions (Nanjing Jiancheng) using the sarcosine oxidase method for Scr and the diacetyl monoxime method for BUN. Absorbance was measured using a microplate reader, and concentrations were calculated from standard curves.

Immunohistochemistry

Paraffin-embedded kidney sections were deparaffinized, rehydrated, subjected to antigen retrieval, and blocked. Sections were incubated overnight at 4 °C with primary antibodies against AQP1, Ki67, α -SMA, p21, or AMD1 (dilutions according to manufacturers' instructions; alternatively, 1:200–1:400). After incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies, immunoreactivity was visualized with 3,3'-diaminobenzidine (DAB). Positive staining was quantified as the percentage of positive area in 20 random fields per section using ImageJ.

Immunofluorescence

After deparaffinization, antigen retrieval, and blocking, kidney sections were incubated with primary antibodies against target proteins together with segment-specific tubular markers, followed by fluorophore-conjugated secondary antibodies. Nuclei were counterstained when applicable. Images were acquired using a fluorescence microscope, and co-localization across tubular segments was assessed by merged-channel analysis.

Senescence-Associated β -Galactosidase (SA- β -Gal) Staining

Frozen kidney sections (8–10 μ m) were fixed and incubated with SA- β -Gal staining solution (pH 6.0) at 37 °C (no CO₂) overnight. Images were captured under a light microscope, and the SA- β -Gal-positive area was quantified as a percentage of the total tissue area using ImageJ.

RT-qPCR

Total RNA was extracted from renal cortex tissue and reverse-transcribed into cDNA. Quantitative PCR was performed using SYBR Green chemistry to quantify mRNA levels of SASP-associated genes,

including IL6, CCL2, and CXCL1. Gapdh was used as the internal control, and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell Culture and siRNA Transfection

Human proximal tubular epithelial cells (HK-2; Procell, Wuhan, China) were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum (FBS). Cells in logarithmic growth phase were seeded and transfected with siAMD1 or negative control siRNA (siNC) using Lipofectamine 3000 according to the manufacturer's protocol. After 6 h, the medium was replaced. For rescue experiments, Spd was added to the culture medium at 10 μ M and incubated for 24–48 h.

EdU Incorporation Assay

HK-2 cells cultured on coverslips were incubated with 10 μ M EdU for 2 h, fixed, permeabilized, and stained using an Apollo reaction cocktail followed by nuclear counterstaining with Hoechst 33342. Five random fields per coverslip were imaged, and the proportion of EdU-positive cells was quantified using ImageJ.

Statistical Analysis

Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism 9.5. Comparisons among multiple groups were conducted using one-way ANOVA followed by Tukey's post hoc test. A two-tailed p-value < 0.05 was considered statistically significant.

Declarations

Supplementary Materials: Listed in Supplementary Materials.

Author Contributions: Conceptualization: B.M. (Baiwei Mao) and Z.Z. (Zhihuang Zheng). Methodology: B.M., Z.Z., W.F. (Wenxin Fu) and G.C. (Guozhe Cheng). Validation: Z.Z., L.W. (Lijun Wang), J.B. (Jinfang Bao) and J.L. (Jun Liu). Resources: Z.Z., J.L., X.L. (Xiaohua Liu), H.Z. (Hongbin Zhan) and M.P. (Miao pan). Data curation: B.M. and Z.Z. Writing—original draft: B.M. Writing—review and editing: Z.Z. and J.L. Visualization: B.M. Supervision: J.L. Project administration: J.L. Funding acquisition: J.L. and Z.Z. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee (IACUC)/Ethics Committee of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine (protocol code 2021AW016).

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets analyzed in this study are publicly available in the Gene Expression Omnibus (GEO) under accession numbers GSE165876 and GSE180420. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

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