The protective effect of preconditioning in cisplatin-induced acute kidney injury is determined by mRNA-independent alterations of the proteome microenvironment

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Abstract.

Acute kidney injury (AKI) is one of the most common renal disorders leading to significant morbidity and mortality. Unfortunately, strategies to prevent or treat AKI are lacking. In recent years, a series of preconditioning protocols has been shown to be effective in inducing organ protection in rodent models. Here, we characterized two of these interventions – caloric restriction and hypoxic preconditioning – in a mouse model of cisplatin-induced AKI and investigated the underlying mechanisms by acquisition of multi-layered omic data (transcriptome, proteome, N-degradome and membrane lipidome) and functional parameters in the same animals. Both preconditioning protocols markedly ameliorated cisplatin-induced loss of renal function. Bioinformatic analysis revealed mRNA-independent proteome alterations affecting the extracellular space, tubule mitochondria and transporters. Interestingly, our analyses revealed a strong dissociation of protein and RNA expression after cisplatin treatment that showed a strong correlation with the degree of damage across the cohort. N-degradomic analysis revealed that most posttranscriptional changes were determined by Arg-specific proteolytic processing. This included a characteristic cisplatin-activated complement signature that was prevented by preconditioning. In addition, amyloid and acute-phase proteins accumulated within the cortical parenchyma. Extensive analysis of disease associated molecular patterns suggested that transcription-independent deposition of amyloid P-component serum protein may be a key component in the microenvironmental contribution to kidney damage. In conclusion, this proof-of-principle study provides new insights into the pathogenesis of cisplatin-induced AKI and the molecular mechanisms underlying organ protection by the means of multi-omic-phenotype correlations.

Introduction.

Acute kidney injury (AKI) is one of the most common complications in hospitalized patients and shows a rapidly increasing incidence – especially in the elderly ^{1,2}. Although partial recovery occurs in the majority of patients, AKI is strongly associated with prolonged hospitalization, increase of mortality and progression to end-stage renal disease (ESRD) ^{3–7}. ESRD can also be the consequence of recurrent, minor episodes of AKI during lifetime ⁸. AKI is associated with financial burden to modern healthcare systems exceeding an estimate of 10 billion dollars per year in the US ^{7,9,10}. While pre- or postrenal AKI can be detected and treated rather readily, the actual cellular damage of kidney tissue (intrarenal AKI) is the major determinant of outcome. Several key factors causing intrarenal AKI are known, among these ischemia-reperfusion during injury surgery, and nephrotoxic agent exposure. However, targeted ways to prevent this damage are still not available.

In 1986 Murry et al. showed that the exposure of the myocardium to sublethal levels of ischemia can protect animals from consecutive myocardial infarction – a concept called ischemic preconditioning ¹¹. This effect is not limited to the heart but can also be exploited in other organs such as the kidney ¹². Since then, several renal preconditioning interventions have been characterized primarily in rodent models. Two prominent examples are caloric restriction (CR) and hypoxic preconditioning (HP) that showed substantial protective potential for AKI^{13–15}. Unfortunately, these strategies have not made their way to clinical application so far due to problems with feasibility on the one hand but also reproducibility in the much more complex setting of patients' care on the other hand. Consequently, a more profound knowledge of the molecular key changes in different forms of AKI and the molecular mechanisms underlying preconditioning-mediated organoprotection is essential to uncover refined therapeutic targets in patients.

Here, we demonstrate that hypoxic preconditioning and caloric restriction can be both utilized to prevent intrarenal damage inflicted by nephrotoxic treatment in mice. We then applied multi-layered omics approach to investigate the molecular composition of a protected damaged versus an unprotected damaged kidney to prioritize the most important molecular patterns associated with renal damage. Transcriptomic (mRNAseq) and proteomic analyses revealed a strong dissociation of protein and RNA expression after AKI correlating with the severity of injury. N-Degradomic analyses exposed that most posttranscriptional changes were determined by serine protease-dependent proteolytic processing, including a characteristic complement activation signature.

Results.

To induce acute kidney injury we applied an intraperitoneal bolus of cisplatin to untreated wildtype mice (cis, Fig. 1A). At the same time, we administered cisplatin to mice either preconditioned by caloric restriction¹³ (CR, only 66 % of the averaged daily intake of calories for 28 days) or by incremental hypoxia¹⁶ (HP, 8.3 % O₂ for 2/4/8 h on three following days) (Fig. 1A). Compared to vehicle treated mice (veh), cisplatin treated mice (cis) showed a significant weight loss after 72 h hours (one-way ANOVA and Bonferroni's Multiple Comparison Test p-value < 0.001). Calorically restricted (CR) and hypoxia (HP) treated animals did not lose weight significantly 72 h after AKI (Fig. 1B, Fig. S1A) (one-way ANOVA and Bonferroni's Multiple Comparison Test p-value < 0.05). Consistent with a protective effect of our preconditioning strategies, blood urea nitrogen (BUN) and plasma creatinine were significantly higher in non-preconditioned animals after cisplatin administration than in preconditioned animals (Fig. 1C, D). Plasma values of creatinine and BUN showed a strong correlation with plasma values of Cystatin C in a different cohort supporting the loss of kidney function due to cisplatin treatment and prevention by preconditioning (Fig. S2). Histologically, kidneys of cisplatin treated control mice showed a significant deposition of PAS positive material in the interstitium (Fig. 1E). In addition, nuclear pyknosis indicative of cell death and intratubular protein casts were observed in the kidneys treated with cisplatin. In hypoxia treated animals (HP), kidneys showed a significantly better histological appearance with less nuclear pyknosis and less casts. Calorically restricted (CR) mice showed a histological outcome after cisplatin treatment, which was not notably different from kidneys obtained from vehicle treated animals (Fig. 1E). Semi-quantitative analysis of histological damage showed a protective effect, particularly in CR animals (Fig. S3A). To further validate histological damage we have also included immunostainings of KIM1, a marker for renal damage¹⁷, that suggested a stronger renal damage in cis > HP > CR > veh (Fig. S3B). Analysis of regulated cell death as determined by TUNEL assay demonstrated a high number of TUNEL positive cells in the kidneys of animals only treated with cisplatin, significantly less after hypoxic preconditioning and none in calorically restricted or vehicle treated animals (Fig. 1F). These results demonstrate that preconditioning interventions have the potential to ameliorate cisplatin-induced intra-renal damage.

In order to characterize the molecular changes induced by cisplatin-toxicity and their modulation by preconditioning we performed quantitative label-free proteomic analysis of the renal cortices of the same mice we used for the analyses mentioned above and identified more than 6000 proteins. 4733 proteins could be identified across all samples - (see methods for details, see table S1 for detailed results). Using hierarchical clustering for Euclidean distance, we found a clear separation between vehicle treated and cisplatin

treated kidneys (Fig. 2A). We also found that in principal component analysis (PCA), cisplatin and vehicle treated samples were clearly distinguishable. In contrast, proteomic profiles of vehicle and cisplatin treated kidneys after caloric restriction were very similar (Fig. 2B). Then we analyzed the effect of cisplatin on kidney tissue as compared to vehicle treatment. Comparison of protein abundance between both conditions with stringent parameters ($s0=0.1$ and $FDR < 0.01$) identified 1448 significantly changed proteins. 636 proteins were increased, and 812 were decreased after cisplatin treatment (Fig. 2C). We found many proteins known to increase with acute kidney injury, among these Ng2 (gene symbol: *Lcn2*¹⁸) and alpha fetuin-a (gene symbol: *Ahsg*¹⁹). A GO term enrichment ($FDR = 0.02$) analysis revealed, that proteins related to extracellular matrix and acute phase reaction were increased after cisplatin exposure, consistent with an inflammatory and fibrotic response in the tissue (Fig. 2D). Moreover, proteins associated with transmembrane receptor protein kinase activity were increased, consistent with protein alterations affecting intracellular response machineries (Fig. 2D). Conversely, proteins associated with the excretion of ions, amino acids and metabolites were decreased in the dataset, as well as proteins associated with mitochondria (Fig. 2D).

“Extracellular matrix” is an ambiguous term in which multiple biological entities are lumped together (e.g. secreted proteins and known basal lamina and “matrisome” associated proteins)^{20,21}. Among the upregulated proteins, we found core matrisome proteins, but also secreted matrisome associated proteins as well as secreted proteins not related to the matrisome (e.g. amylase, lysozyme and others, Fig. 2E). Among these secreted proteins, we found an increase in particular smaller proteins that are more easily filtered and taken up by endocytic mechanisms in the proximal tubule²² (Fig. 2F).

Next, we performed hierarchical clustering analyses to define global proteomic shifts across the four treatment groups (Fig. 3A). 6 major clusters, each with more than 10 proteins, could be defined (Fig. 3B). Three clusters had protein patterns increasing with extent of damage (protein expression $veh < CR < HP < cis$). These clusters were significantly enriched for several distinct processes such as complement, extracellular matrix generation, and chemotaxis (Fig. 3C). One cluster showed a decreased protein expression with the extent of damage (protein expression $veh > CR > HP > cis$). This cluster was largely enriched for transmembrane proteins and proteins involved in proximal tubule function such as sodium transport and amino-acid transport. One cluster containing 13 proteins (cluster 5) peaked specifically in kidneys of CR preconditioned animals, which were almost completely protected from cisplatin induced damage. This cluster was enriched for proteins involved in fatty acid biosynthesis, indicating that the induction of these proteins may be involved in the protective capacity of caloric restriction.

To further investigate whether these changes were relevant, we performed targeted lipidomic analysis by Nano-Electrospray Ionization Tandem Mass Spectrometry with direct infusion of lipid extracts from treated mouse kidneys (*Shotgun Lipidomics*). The levels of different glycerophospholipid subspecies (phosphatidylcholins, PC, phosphatidylethanolamines, PE, phosphatidylinositols, PI, phosphatidylserines, PS) were determined by lipid class-specific neutral loss and multiple precursor ion scanning (for detailed results, see suppl. table S2). This analysis revealed a loss of membrane lipids during cisplatin induced injury. This loss of lipids was lower after CR suggesting that a loss of membrane integrity is associated with cisplatin induced injury, which can be abolished by CR (Fig. 3D).

Prominent alterations in mRNA expression have been determined by high-throughput analyses of kidneys after the induction of different modes of AKI ²³. Yet, mRNA expression does not necessarily and always predict protein abundance ²⁴. Thus, we asked whether the extent of dysregulated proteins was largely due to the expression of altered transcripts. To this end, we performed analyses of the transcriptome from the same kidneys as used for the proteomics analyses using next generation sequencing (NGS) – see table S3 for detailed results. Employing t-SNE clustering of the individual transcript levels, we found that samples of vehicle treated (veh) mice and samples of cisplatin treated mice after caloric restriction (CR) were closely related and both showed similar differences in comparison to the samples of unpreconditioned cisplatin treated animals (cis), consistent with our proteome data (Fig. 4A). Of note, the fold-changes (e.g. in cis vs veh) were less prominent as determined by proteomics analysis (Fig. S4A, B). Enrichment analysis determined similar pathways being regulated as compared to the proteome (Fig. S4C, D). Next, we performed a correlative analysis between mRNA and protein copy number level for every individual protein-mRNA pair. The distribution of mRNA-protein correlation coefficients of all individual protein-mRNA correlation coefficients revealed that transcript copy numbers only moderately determined protein abundance in all treated samples (Fig. 4B) as commonly observed ²⁵. Interestingly, when analyzing each treatment group separately, we found that correlation between transcripts and proteome levels was highest in CR (Fig. 4C) ($R = 0.57$), significantly lower in HP ($R = 0.54$) and much lower in cis ($R = 0.47$). To examine the relationship of a low correlation coefficient of the transcriptome and proteome and the severity of kidney damage, we plotted the correlation coefficient against the individual creatinine value in every mouse analyzed. Notably, we observed a very strong and significant connection between the correlation coefficient R and $\log_2(\text{creatinine})$, $R = -0.98$ (Fig. 4D), that could also be recapitulated by usage of the values for BUN ($R = -0.96$) (Fig. 4 E). Both creatinine and BUN showed strong correlations with Cystatin C (Fig. S2), which is another well-known biomarker for AKI ¹⁷. The connection between the correlation coefficient R and $\log_2(\text{creatinine})$

suggested that - with increased severity of kidney damage - transcriptomic data becomes less predictive of protein abundance indicating that posttranscriptional changes must play an important role in the response to injury.

Posttranslational regulatory mechanisms, such as proteolytic cleavage reactions by proteases or the proteasome, have been described as a major regulator of the posttranscriptional landscape in different scenarios of cell death, including AKI ^{26,27}. Mass spectrometry-based N-degradomics enables identification and quantification of protein N-termini and proteolytic activity on a proteome-wide scale ²⁸⁻³⁰. We used the established Terminal Amine Isotope Labeling (TAILS) ^{30,31} protocol to perform an N-degradomic analysis of the kidneys of the same animals used for proteomics and transcriptomics characterization as described above. TAILS indiscriminately enriches all protein N-terminal peptides including those generated by proteases or those blocked by endogenous N-terminal acetylation. In total, we identified 1865 N-termini in two complementary triplex labeling experiments that probed altered cisplatin-induced processing and the protective effect of CR (by comparing cis, CR, and vehicle; n=3; one experiment was excluded from data analysis due to an error during sample preparation), and HP (comparing cis, HP, and vehicle; n=4) (Fig. S5A, table S4 for detailed results). Cis/veh ratios of both experiments correlated well indicating technical reproducibility (Fig. S5B). Positional annotation discriminated 1306 “expected” N-termini (matching to position 1 of the predicted protein models or known processing sites such as N-terminal methionine excision, removal of predicted signal-, transit- or pro-peptide sequences) from 559 N-termini mapping to “unexpected” sites within the corresponding protein models, mostly representing dimethylated protease generated neo-N-termini (Fig. 5A). Ratiometric analysis of the different conditions in the dataset found that cisplatin treatment induced a skewed ratio distribution particularly among unexpected N-termini, with similar results for kidneys from CR (cis/CR, cis/veh, Fig. 5A) and HP pretreated mice (cis/HP, cis/veh, Fig. 5B), indicating increased proteolytic activity in damaged kidneys. Analysis of positional amino acid frequency matrices (motifs) can be used to infer dominant proteolytic activities ³⁰. Here, motif analysis showed that in the CR experiment, cisplatin treatment increased mainly termini starting with S or T predominantly resulting from cleavage after a basic residue (K, R and H; Fig. 5C). This was also found in the HP dataset (Fig. 5D), suggesting a cisplatin-induced activity of arginine and lysine-specific proteases such as trypsin-like serine proteases. To test whether these alterations were independent of total proteome changes, we tested whether total proteome fold changes in cisplatin-induced damage correlated with the fold change of the respective cleavage sites within the protein. We observed a strong and very significant correlation between proteome expression and N-termini abundance (Fig. 5E), indicating that also cleaved fragments abundance largely reflected protein abundance. Consistently, we found that there was a very weak negative correlation between N-termini abundance and the

protein-mRNA correlation coefficient described above (Fig. S5C). Interestingly, we observed that several termini that were increased in both pretreatment conditions were chiefly extracellular proteins, suggesting that the majority of the observed proteolytic processes induced by cisplatin treatment occurred extracellularly (Fig. 5F). We also analyzed proteasome activity as chymotrypsin-like activity in the kidney cortex. There was no significant difference in proteasome activity between the samples (Fig S6). This suggests that the observed degradomic alterations are not part of a general, unspecific response regulating proteolytic protein decay, but rather part of specific extracellular proteolytic signaling processes.

Given the predominant increase extracellular processes (Fig. 2D), we correlated the individual proteins obtained from the proteomic data with the parameters of kidney function (creatinine/BUN) revealing a broad distribution of correlation coefficients for individual proteins (Fig. 6A). Reactome term enrichment analysis revealed a strong correlation of the creatinine level with proteins related to extracellular matrix synthesis, serum proteins and complement activation (Fig 6B). Complementary and consistent with these findings, N-degradomic analysis showed a strong proteolytic activation of the complement cascade demonstrating specific cleavage of complement components C3 and C4 as well as activation of upstream regulators of the lectin-binding, alternative and classical pathway (Fig. 6C, table S5).

Finally, we aimed to expand our dataset to unknown disease processes. Damage associated molecular patterns (DAMPs) have been suggested to correspond to acute cell death and tissue damage³². Therefore, we analyzed our proteomic dataset - which clearly reflects these damage processes - to determine novel patterns of protein-based DAMPs. However, out of 72 known DAMPs compiled from the literature (Table S6, Fig. 7A) only 27 of them could be identified in the proteomic dataset and their mean correlation with the renal damage parameter plasma creatinine was very moderate over the animal cohort (average Spearman or Pearson correlation of 0.2). We then expanded the list of known DAMPs to “suggested DAMPS” by inclusion of functionally related protein families in the Reactome database³³. Using this strategy, we found that the Reactome pathway “Amyloid fiber formation” and especially the protein APCS, a circulating plasma protein suggested to be associated with binding of apoptotic cells and tubule formation in vitro^{34,35}, correlated most strongly with the degree of kidney damage (Fig. 7B). The correlation on the mRNA level was much less prominent (Fig. 7C), and APCS was found to be only moderately increased on mRNA level (Fig. S7A). Consistently, accumulation and binding of APCS to tubule structures was also shown in immunohistochemical staining of cisplatin-treated kidneys while in vehicle treated kidney only diffuse background staining was shown (Fig 7D). Ultrasensitive mass

spectrometry confirmed that APCS protein was found in both PAS positive tubules as well as PAS positive protein casts (2 of 2 identified peptides were unique) (Fig. S7B, C).

Discussion.

Preconditioning-mediated protection from AKI holds the promise to reveal novel targets to prevent AKI in the clinical setting. In this study, we have shown the potential of two modes of preconditioning (CR and HP) in the prevention of cisplatin-induced AKI. Cisplatin induced a specific signature on protein abundance with an increased expression of known injury molecules and extracellular matrix components, and a decrease of specialized tubule epithelial proteins. Notably, both modes of preconditioning were strongly protective. CR ameliorated cisplatin-induced damage to an extent that made kidneys of these animals almost indistinguishable from healthy vehicle treated controls. One exception was the finding that kidneys from calorically restricted mice had a higher abundance of proteins promoting fatty acid synthesis – a metabolic pathway that has interestingly already been linked to stress resistance in other models such as nematodes^{36–38}. Analysis of glycerophospholipids revealed a loss of membrane lipids due to cisplatin treatment that is lower after CR. This suggests that loss of membrane integrity (likely induced by complement activation) is a key mechanism that is associated with cisplatin induced injury. In fact, this can be nearly abolished by endogenous fatty acid biosynthesis that is induced by CR (Fig. 8).

This first multi-layered analysis of a proof-of-principle dataset of kidneys undergoing AKI revealed that a significant part of the damage response is probably not mediated on the transcriptional level but occurs post-transcriptionally. In fact, the dissociation of mRNA and proteome levels was strongly connected with the creatinine level in the individual animal – with a surprisingly high correlation coefficient ($R > 0.95$). Degradomics analyses revealed that motif-specific extracellular regulated protease activity is a significant contributor to the dissociation of mRNA and protein level. This included, for example, site-specific proteolytic activation and deposition of various complement cascade components converging in C3 activation that are comprehensively quantified within one mass spectrometry experiment. Consistently with a disease-propagating role of this pathway, genetic ablation of complement C5 was shown to be protective in cisplatin-induced AKI, a finding partially attributed to induced release of the anaphylatoxin C5a³⁹. A major role for extracellular signaling is also supported by bioinformatics analysis of DAMP-associated pathways. DAMPs are known to be involved in the intracellular regulation of cell death (such as the NFkappa B pathway, and the TGF related pathway)³². The most creatinine-correlated DAMPs term, however, was extracellular amyloid fiber formation, and serum amyloid P (APCS) was its most prominent

component. APCS is suggested to bind to apoptotic cells, but has also been proposed to increase tubule formation and regeneration in vitro ³⁴. APCS deposition is also described to be inhibited by both HP ⁴⁰ and CR ⁴¹ interventions in neurodegenerative diseases, suggesting an overarching mechanism controlling these aspects of tissue homeostasis. These data expand the commonly known and prominent role in chronic amyloidosis-like diseases of the kidney into this more acute setting.

In chronic kidney disease, extracellular matrix deposition and fibrosis determines decline of kidney function ^{42–45}. In this model of cisplatin-induced AKI, the observed molecular changes occur rapidly and in the extracellular compartment. Notably, they are not reached by changing RNA abundance or production but rather protein quantity. Altogether, this is consistent with the hypothesis that the extent of cisplatin induced AKI – as a variety of regulated cell death processes – is determined and maintained by “outside-in” signaling and the molecular composition of the extracellular microenvironment that mediates inflammatory processes ^{46,47,48}. Taking all the findings together one possible mechanism could be a complement induced lysis of cell membranes leading to an inflammatory microenvironment and cell death recruiting APCS by a release of calcium⁴⁹. CR seems to prevent AKI by endogenous stabilization of cell membranes (Fig. 8). Since APCS and other small molecules are also found in the tubule lumen, a contribution of endocytotic mechanisms to kidney damage cannot be excluded. Changes in glomerular filtration rate, going in line with AKI, have already been linked to fluid shear stress and a consecutive increase of endocytosis in proximal tubular cells ^{50,51} and will require further investigation. In addition, a limitation of this study is that it yet does not distinguish between regenerative and detrimental pathways and the baseline omics landscape of the preconditioned kidney requires further investigation.

In conclusion, we provide an in depth multi-omic driven characterization of cisplatin induced kidney injury and preventive strategies that ameliorate renal damage. Although some of the pathways such as complement activation have been described in humans ^{52,53}, the transferability of the findings in this proof-of-principle study to other models and, ultimately, human AKI, needs to be demonstrated. The obtained phenotype-driven computable datasets show that novel drug targets will ultimately have to block key microenvironmental shifts at a systems level, including extracellular protein deposition and proteolytic activity, in order to improve the outcome of AKI.

Methods

Animal procedures.

All mice were supervised daily after intraperitoneal injection and all procedures were approved by the LANUV NRW (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, State Agency for Nature, Environment and Consumer Protection North Rhine-Westphalia; VSG 84-02.04.2013.A158). 19 – 21-week-old, male C57Bl6 wildtype mice originally obtained by Charles River (Sulzfeld, Germany) were kept under specific pathogen free conditions with a daily 12 hour light and 12 hour dark rhythm. In one cage there were up to 5 mice at the same time. All mice received ad libitum water and all mice except calorically restricted mice received ad libitum standard food provided by sniff (Soest, Germany). Calorically restricted mice received 66 % of the usual daily intake (3 g per day) for 28 days, according to Mitchell *et al.*¹³, prior to cisplatin injection. Hypoxic preconditioning was performed by reduction of oxygen to 8.3 % in a hypoxia chamber for 2 h, 4 h and 8 h on three consecutive days based on the protocol of Bernaudin *et al.*¹⁶. Intraperitoneal cisplatin injection (20 mg/kg bodyweight) was performed 1 h after the hypoxic preconditioning protocol was finished. Cisplatin [1 mg/ml] was provided by the central cytostatic drugstore of the University Hospital of Cologne. Standard sodium-chloride solution (0.9 %) was used for the vehicle-control group. Intraperitoneal injections were performed under isoflurane-insufflation. 72 h hours after injection the mice were anaesthetized by intraperitoneal ketamine/xylazine-injection. Blood was obtained by cardiac puncture followed by a cardiac perfusion with phosphate buffered saline. Right kidneys were cut into two equal sections. One section was embedded and frozen in Tissue-Tek® O.C.T.™ compound (Sakura, Leiden, Netherlands). The other section was fixed in 4% formaldehyde and subsequently embedded in paraffin. Left kidneys were cut into two equal sections. One section was macroscopically divided into cortex and medulla for proteomic analysis. The other section was divided again into another two equal pieces. All parts of the left kidneys were snap frozen in liquid nitrogen for mRNAseq and N-degradome-analysis.

Functional data and statistics.

Plasma creatinine, blood urea nitrogen and Cystatin C were measured by the central laboratory of the University Hospital of Cologne using a Cobas C 702 (Roche Diagnostics, Germany). Statistical analysis was performed by prism 5 (GraphPad, USA) using one-way ANOVA and Bonferroni posthoc-test. Unless otherwise indicated a P-value < 0.05 was considered significant.

Histopathology

Formalin-fixed tissue was cut into 2 μ m slices and Periodic acid-Schiff (PAS) staining was performed. Furthermore another 2 μ m thick paraffin sections were stained using the anti-APCS antibody (LS-B11917, LifeSpan Biosciences, Inc., USA) according to the manufacturer's protocol in a 1:500 dilution. Anti-KIM-1 staining was performed by using 2 μ m paraffin sections using the Anti-KIM-1 antibody (ab78494, Abcam Cambridge, UK) according to the manufacturer's protocol in a 1:400 dilution. Pictures were taken by a slidescanner SCN4000 (Leica, Germany) in a 20x magnification. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed on 2 μ m paraffin sections using the DeadEnd Fluorometric TUNEL System (Promega, USA) according to the manufacturer's protocol. Images were acquired using an Axiovert 200M microscope equipped with a Plan-Apochromat 20x/0.8 objective and an AxioCamMR camera (all from Carl Zeiss MicroImaging GmbH). The Microscope was operated using Axiovision software (version 4.8.2).

Semiquantitative analysis of tubular damage of each kidney used for omic-analysis was performed by two different experienced nephrologists in a blinded manner. A composite score to classify tubular damage was used as previously described⁵⁴ and the mean for each treatment group was plotted.

RNA isolation and sequencing

RNA isolation of total RNA was performed by using commercial homogenization (Bio 101 FastPrep FP120-120 V, Savant, Midland, MI, USA) and the RNeasy mini kit (Qiagen, Hilden, Germany). RNA sequencing was done by the Cologne Center for Genomics using the Ribo-Zero™ Human/Mouse/Rat preparation kits and the Illumina® TruSeq® Stranded Total RNASamples (Illumina, USA).

RNA-seq processing

Paired end total RNA-seq data was mapped to the mouse genome (mm10) using STAR aligner (v.2.5) with the default options and splice junction annotation from Ensembl (GRCm38.83). Count value for each gene was obtained using featureCount command from subread program (v.1.5) with the following options: paired end (-p) only considering primary alignment (--primary) and strand-specific for reverse forward protocol (-s 2; -S fr). For normalization, we used the transcript per millions (TPM) as defined by Li *et al.*⁵⁵.

Reverse Transcription and Quantitative Real-time PCR (qPCR) Analysis

The already isolated RNA was used. 0.5 μ g of total RNA was used as input. The cDNA synthesis was performed using the high-capacity-kit cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, USA). The cDNA products were further diluted 1:1 and

used as qPCR templates in a triplicate experiment. qPCR was performed as previously described⁵⁶ using PrimeTime[®] Mini qPCR assays by IDT DNA Technologies (Skokie, Illinois, USA) by using the manufacturers protocol for Lcn2 (Mm.PT.58.10167155), C3 (Mm.PT.58.17325540), Apcs (Mm.PT.58.9146631.gs) and C4b (Mm.PT.58.42828296.g). qPCR was performed by using a 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, USA). All samples were normalized by the expression levels of the housekeeping genes ActB and HPRT. Statistical analyses were based on calculated $2^{-\Delta\text{CT}}$ -values using a two-tailed Student's t-test.

Sample preparation for proteomics analysis using LC-MS/MS.

Samples were prepared as previously described⁵⁷. In brief, renal material was homogenized and sonicated in a buffer containing 8M urea, 100mM Ammonium bicarbonate, and protease inhibitor (roche). Samples were reduced with DTT and alkylated using IAA. Then, proteins were digested using trypsin at a 1:100 w/w ratio over-night. Then, peptides were cleaned up using stagetips⁵⁸. Finally, nLC-MS/MS was performed on a QExactive plus (Thermo) Orbitrap machine coupled to a nLC device (Proxeon) as previously described⁵⁹. A 4h gradient was used for the separation of the peptides.

Laser capture dissection microscopy and tissue staining

Laser capture dissection microscopy was performed as previously described⁶⁰. In brief, paraffin embedded tissue was cut into 10 μm slices and Periodic Acid Schiff-(PAS)-staining was performed. Afterwards, the selected regions were captured using a laser microdissection microscope (LMD7000, Leica, Wetzlar, Germany). Two areas in cisplatin injury were defined: area 1: PAS-positive proximal tubules and area 2: PAS-positive tubular casts. Proximal tubules of control animals (veh) served as negative control. Microdissected tissue was captured in 25 μl of a buffer containing 5 % SDS and 10 mM Tris. Sample preparation was performed using the ultrasensitive sample preparation method⁶⁰.

Bioinformatic analysis of proteomic data.

Raw data were searched against a uniprot database (Jan 2017, reference proteome) and processed using MaxQuant v 1.5. with the LFQ (label-free quantification), iBAQ and match between runs option enabled. PSM, peptide and protein FDR were set as 0.01. Protein-groups output was further analyzed in R or in Perseus v 1.5.5.3⁶¹ as previously described⁵⁶. In brief, contaminants and reverse hits, as well as proteins only identified by site were removed. Protein label-free quantification values were $\log_2$ transformed and filtered for the presence of at least 3 valid values per treatment group. Significance cutoffs were determined

using a permutation based false discovery rate (FDR) approach with fudge factor (parameters given in the figure legend) as previously described by Tusher et al.⁶². GO terms and uniprot keywords were annotated using the Perseus annotation package and were subsequently analyzed for overrepresentation using a Fisher's exact test with correction for multiple testing using Benjamini Hochberg FDR. Correlation coefficients were calculated in R and functional data were plotted in R-studio using the ggplot2⁶³ and cowplot packages.

Proteasome activity assay.

25 µg of renal cortex from 4 animals of each treatment group (veh/cis/HP/CR) were resuspended, as previously described⁶⁴, with 50 µl proteasome buffer. Subsequently, cytosolic extracts were generated by centrifugation at 14,000 g for 10 min at 4°C. After determining protein concentrations with a commercial Bradford Assay (Bio-Rad), 25 µg of protein per sample were loaded on a black fluorescence microplate. Then, 60 µl of the proteasome buffer and 0.125 µg/µl of fluorogenic substrate were added to each well. We used Z-Gly-Gly-Leu-AMC (bml-zw8505-0005, ENZO Life Science) as a fluorogenic substrate, which releases its highly fluorescent product, the fluorophor AMC (7-Amino-4-methylcoumarin), upon chymotrypsin like protease activity⁶⁵. Fluorescence (excitation wavelength of 380 nm and emission wavelength of 460 nm) was continuously recorded for 2 h every 5 min by an EnSpire Multimode Plate Reader (PerkinElmer) set at 37°C. If the signal was saturated, the recording stopped. Technical duplicates were incubated additionally with the proteasome inhibitor MG132 (10 µM, Calbiochem) prior to the measurement. Proteasome activity was determined for each sample by quantifying the relative slope of the increase in its fluorescence intensity as a fraction of 1.

Integration of proteomics and transcriptomic data.

Data preprocessing: From the 16 different samples, the proteomic data obtained was expressed as intensity based absolute quantification (iBAQ), while on the transcriptomic dataset it was expressed as transcripts per millions (TPM). As a first step, all zeroes were regarded as missing values (NaN) and the remaining measurements were transformed as the log₂ of the original values. This was applied on both the proteomic and the transcriptomic datasets. Then, and only in the transcriptomic dataset, all values whose log₂ was below zero were also regarded as missing values, since they can be considered below the limit of quantification. Finally, we removed the genes or proteins which only had measurements in one of the conditions or which did not have measurements in the control group, as they were not usable in the differential expression analysis.

Data representation: All plots and data analysis were performed in R. For the t-Stochastic Neighbor Embedding plots (t-SNE), we used the algorithm as implemented in the package Rtsne⁶⁶ as described by van der Maaten *et al.*⁶⁷. The heatmaps and hierarchical clustering were obtained using the package pheatmap⁶⁸. The remaining plots were generated by self-coded functions using the standard library ggplot2⁶³

Differential expression analysis: For both the proteomic and transcriptomic datasets, the differential expression analysis compared the cisplatin treated groups (cis: only treated with cisplatin, HP: cisplatin and hypoxic preconditioning and CR: cisplatin and caloric restriction) against the control group. On each of the comparison groups, a linear model was fitted. Each fit followed the computation of the estimated coefficients and standard error of each gene or protein. Finally, the logodds, moderated t- and F-statistics of the differential expression were calculated using the empirical Bayes moderation of the standard errors towards a common value. The whole analysis was performed using the R package limma⁶⁹.

Gene set analysis: When performing the gene set analysis (GSA), first we mapped all the genes and proteins to their related gene ontology (GO) terms by using UniProt web service⁷⁰. With the t-values obtained from the differential expression analysis as gene set statistics, we ran the GSA using the Platform for Integrated Analysis of Omics data (piano)⁷¹. The analysis was performed using three different gene-level statistics, namely: mean, median and sum. From these, the consensus scores were extracted for the distinct-directional regulated genes/proteins using the same package. This enabled us to retrieve the most significant up- and down-regulated gene-sets (GO terms) of the different treatments with respect to the controls on both the transcript and protein levels.

Protein-transcript correlation analysis: In order to compute the correlation between the transcriptomic and proteomic readouts, we first mapped the genes (as ENSEMBL gene IDs) to the corresponding protein IDs (as UniProt AC). After removing genes with missing values, unmapped names and taking only the intersection between entries on both datasets, we obtained a list of 2510 elements. Then for each entry of the list, we computed the correlation between the protein and transcript levels across all the samples. Finally, to assess the significance of these correlations, we computed the corresponding p-values from the Z-scores obtained by testing each correlation value against the whole distribution and also against a null-distribution (obtained by resampling the labels of the protein measurements 1000 times).

N-degradomic analysis and bioinformatics analysis

Enrichment and identification of protein N-termini: Proteins were extracted from renal cortex as described ³⁰. Enrichment of N-terminal peptides was performed by Terminal Amine Isotope Labeling of Substrates using different formaldehyde isotopes ($^{12}\text{CH}_2\text{O}$, $^{12}\text{CD}_2\text{O}$ and $^{13}\text{CD}_2\text{O}$) and sodium cyanoborohydride or sodium cyanoborodeuteride for modification and labeling of primary amines by triplex reductive dimethylation as described ⁷². The following labeling schemes were used in two separate experiments: 1) control, light label ($^{12}\text{CH}_2\text{O}$ and NaBH_3CN); cisplatin treatment, medium label ($^{12}\text{CD}_2\text{O}$ and NaBH_3CN); cisplatin treatment after hypoxia-preconditioning, heavy label ($^{13}\text{CD}_2\text{O}$ and NaBH_3CN). 2) Control, light label; cisplatin treatment, medium label; cisplatin treatment after caloric restriction, heavy label. Enriched N-terminal peptides were desalted using in-house packed C18 StageTips ⁷³. Samples were analyzed using an Ultimate 3000 RSLCnano HPLC (Thermo) operated in a two-column setup (Acclaim PepMap 100 C18, particle size $3\mu\text{m}$, ID $75\mu\text{m}$, trap column length 2cm, analytical column length 25cm, Thermo) at a flow rate of 300 nl/min. The nanoLC system was on-line coupled to an Impact II high resolution Q-TOF (Bruker) via a CaptiveSpray nano ESI source (Bruker) with a NanoBooster (Bruker) engaged to saturate the nitrogen gas stream with acetonitrile essentially as described ³⁰.

Data analysis: Spectra were matched to peptide sequences at a false discovery rate of 0.01 using the Andromeda algorithm as implemented in the MaxQuant Software package v.1.5.8.3, using the UniProt mouse proteome (release 2015_10) as database for searches with standard Bruker QTOF instrument settings. Search parameters were set as follows: Semi-specific (free N-terminus) ArgC as digestion enzyme, isotope labeling by light (+28.031300), medium (+32.056407) or heavy (+36.075670) dimethylation of Lys residues and peptide N-termini, Cys carbamidomethylation as fixed and Met oxidation, N-terminal acetylation (+42.010565) or N-terminal pyroGlu formation from Glu (-18.010565) or Gln (-17.026549) as variable modifications.

Further bioinformatics analysis was performed to classify termini. An in-house script was utilized to annotate termini (derived from the modificationSpecificPeptides.txt file of the MaxQuant output) with information from Uniprot.org and sequence windows. Expected termini were N-termini starting with Methionine or within the first two amino acids of a protein, or termini starting within 5 amino acid window (N- and C-terminal) adjacent to an annotated signal-, transit-, or pro-peptide cleavage site. Unexpected termini were defined as termini matching no functional annotation and including protease-generated N-termini labeled with dimethyl. Motifs were generated using 6AA windows surrounding the neo-N terminus using Icelogo ⁷⁴ ($p < 0.05$). Increased termini ($\log_2$ fold change > 1) were compared to natural abundance. N-termini of proteins of interest (such as complement) were manually annotated using uniprot, Topfind ⁷⁵ and wikipathway resources. Matrisome information was obtained

from <http://matrisomeproject.mit.edu/other-resources/mouse-matrisome/>^{20,21} (accessed June 2018) and boxplots were generated using R.

Glycerophospholipid analysis

Glycerophospholipids i. e. phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylinositols (PI) and phosphatidylserines (PS) in mouse kidneys were analyzed by Nano-Electrospray Ionization Tandem Mass Spectrometry (Nano-ESI-MS/MS) with direct infusion of the lipid extract (*Shotgun Lipidomics*): Samples of mouse kidney tissue (n = 4) were homogenized in Milli-Q water (1 mg / 10 µl) using the Precellys 24 Homogenisator (Peqlab, Erlangen, Germany) at 6.500 rpm for 30 sec. The protein content of the homogenate was routinely determined using bicinchoninic acid.

To 5 µl of the homogenate 495 µl of Milli-Q water, 1.875 ml of methanol/chloroform 2:1 (v/v) and internal standards (126 pmol PC 17:0-20:4, 145 pmol PE 17:0-20:4, 120 pmol PI 17:0-20:4, 120 pmol PS 17:0-20:4; Avanti Polar Lipids) were added. Lipid extraction and Nano-ESI-MS/MS analysis were performed as previously described⁷⁶. Endogenous glycerophospholipids were quantified by referring their peak areas to those of the internal standards. The calculated glycerophospholipid amounts were normalized to the protein content of the tissue homogenate.

Raw data deposition

The raw proteomic data are available via PRIDE proteomexchange repository 62 accessible via <https://www.ebi.ac.uk/pride/>. Project accession: PXD005525; Username: reviewer24718@ebi.ac.uk; Password: Jh69OaBF; Project accession: PXD007186; Username: reviewer84100@ebi.ac.uk; Password: tIFbwjPM. RNA-seq data used in this study is available at GEO database under accession numbers: GSE106993 (require token: chuggaswnxevtg).

Authors contributions

M.R.S., M.P.B., K.J.R.H., F.D., C.B.S., S.B., M.H. performed experiments, N.P.-E., C.D., F.D., A.M.M., M.H., A.B., J.S.R., J.U.B., H.G., G.C. and P.F.H., M.M.R. analyzed experiments, F.G., T.B., B.S., V.B., M.M.R. and R.U.M. designed study, J.N.K., P.F.H., R.U.M., M.M.R. contributed new tools, P.F.H., R.U.M., M.M.R. interpreted results, M.R.S., R.U.M., M.M.R. wrote the article.

Conflict of interests.

The authors have no financial or other conflict of interest.

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Figure Legends.

Fig. 1. Phenotyping and functional parameters of a pilot cohort with cisplatin induced injury. A. Schematic illustration of the experimental design (veh: vehicle control, cis: cisplatin treatment; HP: preconditioning by hypoxia prior to cisplatin treatment; CR: preconditioning by caloric restriction prior to cisplatin treatment). B. Relative bodyweight 0 h, 24 h, 48 h and 72 h after treatment. Bars indicate mean with standard error of the mean. One-way ANOVA and Bonferroni's Multiple Comparison test showed a significant difference in cis but no difference for veh, HP and CR between 0 h and 72 h (n=4 per group) after cisplatin treatment. (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxia preconditioning; CR: cisplatin treatment after caloric restriction) C. Plasma creatinine values 72 h after injection as means $\pm$ standard deviation. One-way ANOVA and Bonferroni's Multiple Comparison test over all treatment groups (n=4 per group) showed significant differences indicated by ***: $p < 0.001$ D. Blood urea nitrogen values 72 h after injection as means $\pm$ standard deviation. One-way ANOVA and Bonferroni's Multiple Comparison test over all treatment groups (n=4 per group) showed significant differences indicated by ***: $p < 0.001$ (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxia preconditioning; CR: cisplatin treatment after caloric restriction) E. Periodic acid-Schiff (PAS) staining of the kidneys harvested 72 h after treatment showing different extents of parenchymal damage. (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxic preconditioning; CR: cisplatin treatment after caloric restriction, black arrow indicates pyknosis, white arrow indicates PAS-positive intratubular material, asterisk indicates PAS-positive material in the interstitium); scalebar indicates 200 μ m. F. TUNEL-staining of the kidneys harvested 72 h after treatment. Scalebars indicate 200 μ m. (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxia preconditioning; CR: cisplatin treatment after caloric restriction)

Fig. 2. Quantitative proteomics analysis of cisplatin-induced injury. A. Heatmap and hierarchical clustering of proteins found in the 4 differently treated groups *in vivo* (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxic preconditioning; CR: cisplatin treatment after caloric restriction, LFQ: label free quantification). B. Principal component analysis of all proteomic datasets (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxic preconditioning; CR: cisplatin treatment after caloric restriction; * indicates two samples directly next to each other). C. Volcano plot analysis of vehicle treated vs. cisplatin treated kidneys. Of 4733 proteins, 1448 proteins were changed based on two-tailed t-test with SAM correction for multiple testing ($s_0=0.1$ and $FDR < 0.01$). 636 proteins were increased, 812 were decreased with cisplatin. D. GO term enrichment analysis of proteins increased and decreased in the

comparison cisplatin vs vehicle (Fisher's exact test, FDR 0.02). E. Differential analysis of upregulated proteins associated with extracellular matrix. The boxplot depicts the mean cisplatin induced fold changes of various protein groups. Core matrisome proteins: proteins directly belonging to the matrisome; Matrisome associated: proteins associated to the matrisome, other secreted: secreted proteins that are not associated with the matrisome. All other: all proteins neither secreted nor associated with the matrisome. Midline is median and + is mean. Boxplot box and whiskers are defined according to Tukey (whiskers extend to data points that are less than 1.5 x interquartile range away from 1st/3rd quartile). F. Boxplot analysis of secreted proteins from Panel 2E.

Fig. 3. Comparative clustering analysis identifies global proteome patterns correlating with damage extent

A. Heatmap of mean intensities within a single treatment group (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxic preconditioning; CR: cisplatin treatment after caloric restriction; norm. LFQ: normalized label free quantification). Protein clusters (rows) were determined by maximum distance and are color coded. B. Expression profiles of protein clusters defined in Fig. 3A. Hierarchical clustering of proteins by maximum distance defines six major clusters. C. GO term enrichment in each cluster (Fisher's Exact test, FDR <0.02). D. Targeted lipidomic analysis. Bars indicate mean concentrations $\pm$ standard error of the mean. (* = p-values < 0.05, ** = p-values 0.001 to 0.01, *** = p-value < 0.001, PI: phosphatidylcholin, PI: phosphatidylinositol, PS: phosphatidylserine, PE: phosphatidylethanolamine, veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxic preconditioning; CR: cisplatin treatment after caloric restriction).

Fig. 4. Protein-mRNA correlation in cisplatin-induced injury and preconditioning.

A. t-Distributed Stochastic Neighbor Embedding (t-SNE) clustering⁶⁷ of mRNA-seq datasets of Cisplatin-induced injury and preconditioning. The axes show arbitrary units (t-SNE output). B. Histogram showing distribution of mRNA-protein correlation coefficients for every mRNA-protein pair across the 16 animals in the study. The majority of mRNA-protein pairs have only moderate positive correlation coefficients. C. Scatterplot of protein and mRNA abundance across the 4 treatment groups. Sample-wise protein-mRNA correlation coefficients differ between treatment groups. D. Sample-wise protein-mRNA correlation coefficients correlate strongly inversely ($R = -0.98$) with plasma $\log_2$ creatinine [in mg/dL] of the individual animal. Similar strong correlations were found for BUN. E. Sample-wise protein-mRNA correlation coefficients show a strong negative correlation ($R = -0.96$) with plasma blood urea nitrogen (BUN) [in mg/dL] for each individual animal.

Fig. 5. Proteolytic changes and proteome changes in cisplatin-induced injury and preconditioning.

A, B. Histogram of $\log_2$ transformed ratios within the different treatment

groups. Unexpected protease generated N-termini are depicted magenta, expected N-termini are blue. Green and red boxes highlight the N-termini increased higher than two-fold. C,D Sequence motifs at the cleavage site of cisplatin-induced unexpected N-termini (see Fig. 5A,B). Position weighted matrices show the frequency of the respective amino acid at the position of the cleavage (indicated by an arrow and the black and white bar). Basic residues (K and R) are favoured before the cleavage. E. Scatterplot of mean N-termini regulation vs total protein changes in cisplatin treatment. Correlation coefficient (R) = 0.72. F. Scatterplot of cis/CR und cis/HP ratios determines similarities between protective effects in both datasets. Extracellular proteins are depicted in green. Correlation coefficient (R) = 0.74 for the overall dataset, R = 0.83 for termini from secreted proteins, R = 0.57 for termini from other proteins.

Fig. 6. Extracellular acute phase proteome deposition and complement activation reflect extent of renal injury. A. Correlation of individual proteins with plasma creatinine was calculated and ranked. Proteins were classified by 10 quantiles. The minimum (Q1, strong negative correlation with creatinine) and maximum (Q10, strong positive correlation with creatinine) quantile is depicted. B. GO term enrichment analysis of quantiles of Pearson's correlation coefficient. Fishers exact test, FDR < 0.05. C. Activation status of the complement cascade by integration of N-degradomic and proteomic datasets. Termini and total protein abundances were mapped on a modified version of the wikipathway - identification number: WP545 89636 - "human complement activation". Purple boxes are N-degradomic fragments, all yellow are total proteins. c3d* = unknown, previously undescribed fragment in the region of c3d. The corresponding fragments are listed in Supplemental Table S5.

Fig. 7. APCS is increased in cisplatin-induced injury and correlates with creatinine. A. Correlation of 27 known DAMPs and DAMP-associated Reactome classes found in the proteomic dataset with creatinine were plotted. Amyloid fiber formation was the predominant DAMP category associated with highest plasma creatinine. B. APCS protein correlation with creatinine in every individual animal. C. APCS mRNA correlation with creatinine in every individual animal. D. Immunohistochemical staining of APCS in vehicle and cisplatin treated animals. Scale bar indicates 100 μ m. Appropriate negative controls were performed.

Fig. 8: Schematic illustration of the influence of fatty acid synthesis on prevention of cisplatin injury. Cisplatin induces proteome microenvironment alterations, transcriptome-proteome dissociation and complement activation. Caloric restriction leads to an increased synthesis of fatty acids that inhibit cell death by stabilization of membrane lipids. (arrows indicate increase, anchor indicates inhibition).

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Supplemental Figure Legends.

Fig. S1: Absolute bodyweight after cisplatin-injection. A. Absolute bodyweight 0, 24, 48 and 72 h after treatment. (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxia preconditioning; CR: cisplatin treatment after caloric restriction)

Fig. S2: Pearson correlation of creatinine or blood urea nitrogen with Cystatin C in an independent animal cohort. A. Pearson correlation of creatinine with Cystatin C. (R = correlation coefficient) B. Pearson correlation of blood urea nitrogen (BUN) with Cystatin C. (R = correlation coefficient).

Fig. S3: Quantification of histological damage. A. Semiquantitative analysis of histological damage by scoring of the parameters indicated in the figure (tubular epithelial flattening, tubular loss of brush border, tubular debris, tubular epithelial vacuolization and tubular loss of nucleus) Each kidney used for omic-analysis in this study was scored by two different pathologists in a blinded manner and the mean for each treatment group was plotted. Values indicate means $\pm$ standard error of the mean. B. Anti-Kim-1 immunohistochemical staining. Scale bars indicate 100 μ m. (veh: vehicle treatment; cis: cisplatin treatment; HP: cisplatin treatment after hypoxia preconditioning; CR: cisplatin treatment after caloric restriction).

Fig. S4: Transcriptomic changes in cisplatin-induced injury. A. Density plots for each sample after data normalization. B. Volcano plot showing the differential expression of transcripts between cisplatin and control animals. Dots shown in red are considered non-significant. Transcripts with p-value lower than 0.05 and absolute FC above 2 can be considered significant and are shown in blue. C-D. GSA best consensus scores comparing cis treated mice with respect to control group in the transcriptomic dataset. The plot in C shows the up-regulated gene sets, the plot in D the down-regulated.

Fig. S5: N-Degradome changes induced by cisplatin injury after hypoxia or caloric restriction. A. Strategy for degradomics analysis. HP and a CR dataset were generated using triplex dimethyl labeling for N-degradomic proteomic analysis by TAILS. Three detection channels were used in a triplex experiment: light, medium, heavy. HP: cisplatin treatment after hypoxia preconditioning; CR: cisplatin treatment after caloric restriction. B. Fold change analysis in the N-degradome and proteome. Scatterplot of logarithmized ratios of cisplatin/vehicle in the N-degradome (HP dataset, experiment 2) plotted against the fold changes in the N-degradome in the other dataset (CR dataset, experiment 1). $R = 0.89$ green dots indicate secreted proteins. C. Scatterplot showing protein-mRNA correlation vs N-degradomic signal (mean) revealing no significant correlation. Green dots indicate secreted proteins.

Fig. S6: Proteasome activity in native tissue from acute kidney injury. Proteasome activity was measured in native cortex tissues using a fluorescent assay for chymotrypsin-like proteasome activity. n=4/point. One-way ANOVA and Tukey's multiple comparisons test did not show any significant differences between the treatment groups ($p > 0.05$).

Fig. S7: APCS is upregulated by cisplatin injury and can be found in PAS-positive tubules as well as in tubular casts. A. mRNA expression analysis of LCN2, mC3b, mC4 and APCS. (veh: vehicle treatment; cis: cisplatin treatment, * = p-value < 0.05, *** = p-value < 0.001, bars indicate means). B. Overview about the two different areas selected for laser micro dissection. Scalebars indicate 50 μ m. C. APCS protein expression values displayed by bar graphs. (IBAQ: intensity based absolute quantification, bars indicate mean $\pm$ standard error of the mean).

Supplemental Tables.

Table S1: Proteomic analysis of in vivo alterations of renal damage.

Individual kidney cortices were subjected to preconditioning and cisplatin treatment and analyzed by LC-MS/MS. The excel file describes summarized results and comprehensive measurements for every individual animal of the initial study.

Table S2: Membrane lipidome analysis.

The excel file describes measured concentrations of glycerophospholipids for each individual mouse.

Table S3: Transcriptomic analysis of cisplatin-induced damage.

The excel file describes transcriptomic expression values for every individual animal.

Table S4: N-degradomic analysis of cisplatin-induced damage and preconditioning by hypoxia and CR. The excel file describes annotation for every individual N-terminal peptide. N=4 for HP and n=3 for CR experiment. One experiment containing one set of animals was removed due to MS performance issues.

Table S5: Proteolytic events of complement activation. Complement fragments discovered by N-degradomics were manually annotated using uniprot database and the TopFinder database.

Table S6: List of Damage associated molecular patterns (DAMPs). DAMPs were compiled from the literature.

A **B** **C** **D** **E** **F** **Fig. 1**

Fig. 2

A

B

C

D

Fig. 3

A

B

C

D

E

Fig. 4

Fig. 5

A

B

C

Fig. 6

A

B

C

D

Fig. 7

Fig. 8

veh

cis

positive (a)

positive (b)

A

Fig. S2

A

Fig. S1

A

B

Fig. S2

A

B

Fig. S3

A

B

C

Fig. S5

Fig. S6

A

B

C

Fig. S7