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REVIEW

Gene expression and biomarkers in renal transplant ischemia reperfusion injury

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Summary

The incidence of postischemic acute renal allograft failure (ARF) occurs in roughly 25% of cadaveric donor kidney recipients. This high rate remained virtually unchanged over the last decades despite modification in recipient management and modern immunosuppressive strategies. It has recently been shown that among other reasons, the systemic inflammation in the brain death cadaveric organ donor contributes to subsequent ARF in the recipient. This review focuses on the consequences of ischemia and reperfusion on the cellular level and offers potential solutions for the reduction of ARF. Genome-wide gene expression analysis together with sophisticated biostatistical analysis made it possible to identify several candidate gene products and proteins that may act as specific and sensitive biomarker for renal inflammation and ischemia. These markers may be very helpful in the clinical management of patients with a high *a priori* risk of subsequent ARF such as recipients of marginal donor kidneys. Ongoing clinical trials will evaluate whether immunosuppression of the cadaveric organ donor before organ harvest will have the potential to reduce inflammation in the transplant kidney and subsequently lead to a reduction in the rate of ARF.

Introduction

Clinical background

Postischemic acute renal allograft failure (ARF) occurs in roughly 25% of cases [1]. However, there is no uniform definition of ARF which rather represents a continuum between clear primary function and dialysis dependency. By classifying ARF as at least one post-transplant dialysis, the rate of 25% remained virtually unchanged over the last decades despite better short-term outcomes due to better surgical and medical management of the transplant recipient. ARF is among the best predictors for long-term graft survival. It has recently been shown in a large cohort study of 122 175 patients that ARF is highly associated with reduced long-term outcome [2]. In fact, the hazard ratio for graft failure is almost twice as high in recipients who experienced ARF compared with those without that problem, adjusted for many covariates. For comparison, the hazard ratio of early acute rejection for death-censored graft survival was noticeably smaller.

Although frequently used immunosuppressants from the calcineurin inhibitor class have been accused of causing this high incidence of ARF, this association has never formally been established. In fact, the only larger randomized controlled trial (RCT) comparing initial immunosuppression with the calcineurin inhibitor cyclosporine with anti-thymoglobuline induction found no difference in the rate of ARF, but the duration of ARF until resolution, however, was significantly longer in the cyclosporine group [3]. New immunosuppressant from the mTOR family have recently been evaluated on that very topic and some authors report a higher incidence of ARF in nonrandomized trials [4]. The recent RCT, however, did not show a higher proportion or longer duration of ARF

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in *de novo* renal transplant patients with an initial immunosuppressive mTOR therapy [5].

Thus it remains unclear what specifically increases the propensity for the cadaveric renal allograft to develop ARF after engraftment. It is intuitive that factors occurring before engraftment and eventually reperfusion may play a key role in this setting. Thus, many researchers focused their efforts on unraveling the problem of ARF on the organ donor. Clinical as well as molecular studies have been carried out recently to identify donor risk factors for ARF which may potentially be modified and thus the rate and duration of ARF might be reduced. Other clinical risk factors for ARF include prolonged warm ischemic time during organ harvesting and graft implantation (anastomosis time), but reasonable cold ischemic times (<15 h) contribute little to ARF and graft survival [6].

Besides this procedural-related factors, intrinsic donor factors are main contributors to ARF. The brain death cadaveric organ donor is usually maintained in an ICU and develops all characteristics of a systemic inflammation similarly to severe inflammatory response syndrome (SIRS) of ICU patients. Furthermore, brain death of the donor usually leads to the development of a central diabetes insipidus causing urinary loss of free water which is usually not adequately replaced in the ICUs because colleagues with little experience in donor management are reluctant to substitute more than a liter of free water per hour. Dehydration and brain death also lead to arterial hypertension which makes the use of vasoconstructive drugs necessary. All these mechanisms are well known to cause renal hypoperfusion and inflammation, key risk factors for subsequent ARF.

These systemic processes severely affect the prospective transplant kidney on a cellular and molecular level. On a morphological basis, it is impossible to distinguish donor kidney source and quality, but on the molecular level a discrete set of transcripts is activated in deceased donor organs. The aim of this review was to present and discuss recent findings of cellular and molecular events occurring before and during ischemia and reperfusion of the transplant kidney. Furthermore, potential strategies to prevent these molecular processes with the potential to reduce the rate and duration of ARF will be discussed briefly. This is especially important because due to the global shortage of cadaveric donor organs, an increasing rate of so called marginal donor organs are nowadays accepted for transplantation which would have been refused before. Thus, we need to face the possibility of even increasing rates of ARF in the near future if no valid prevention or intervention is being developed.

Analysis of ischemia reperfusion injury on a cell-wide level

Unraveling cellular processes linked to ARF may provide insight into relevant mechanisms linked to the clinical picture. Significant experimental advances have been made for analyzing pathophysiologic states involving a diverse spectrum of intracellular processes as also supposed for alterations encountered along renal reperfusion injury. Main technologies include differential gene expression analysis and proteomics, i.e. recording relative mRNA concentration profiles or protein abundance profiles. Studying gene expression and protein concentration profiles on a cell-wide level has changed the experimental approach towards decoding cellular processes. The traditional, deductive approach has been tackling a strictly finite number of genes or proteins, selected based on given hypotheses with respect to the disease under analysis. The 'omics' revolution has changed this procedure: First, an explorative analysis including a wide spectrum of genes/proteins is performed, subsequently using this broad spectrum of information for generating novel hypotheses, usually supported by bioinformatics. Key players identified in the course of these procedures subsequently have to be verified for assuring their validity and reducing overall false-positive rates.

Both, hypothesis-driven candidate gene/protein analysis, as well as genomics/proteomics approaches have been applied to analyze acute renal failure and chronic kidney disease, as well as the function of the transplant organ. Reviews on proteomics in renal disease have been recently published by Thongboonkerd [7,8]. Gene expression studies have been performed for a broad spectrum of kidney transplant-related issues including acute rejection [9], chronic rejection [10], delayed graft function [11], impact of donor age [12], as well as tolerance [13], just to give examples.

This review provides an overview on the present status of gene expression studies along renal transplant organs with particular focus on early events after transplantation. Results from differential gene expression in ischemia reperfusion injury utilizing animal model systems are cross-compiled with expression data derived from human biopsy samples. Major functional protein categories are derived and discussed in the context of short- and long-term graft function.

The review is complemented by an overview on protein markers associated with reperfusion injury. A set of candidate markers has emerged showing promising diagnostic potential for early detection of acute renal failure – potentially of use in the transplant situation for assuring timely therapeutic intervention. Finally, an outlook on experimental therapy approaches aimed at detaining the

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severity of reperfusion injury in the context of post-transplant acute renal failure is given.

Differential gene expression in transplant kidneys

Analysis workflows

First and critical issue in analyzing differential gene expression is availability of proper sample material, and furthermore stringent reproducibility of subsequent RNA extraction and hybridization. Our group has achieved reproducible results when using fresh frozen biopsies whereas formalin-embedded samples have shown major variability in RNA content and quality. Further issue is preparation of sample material, either homogenizing full biopsy sections, or performing, e.g. laser capture microdissection for selecting glomerula and tubuli/interstitium for analysis of gene expression on the level of compartments.

A variety of array technologies have been successfully established. Two-color platforms, including cDNA arrays, where sequences of some hundred base pairs in length are mechanically spotted to microarrays [14], spotted oligonucleotide arrays with sequence stretches up to 70 oligonucleotides [15], as well as one-color platforms (GeneChips, Affymetrix, Santa Clara, CA, USA) where short sequences are *in situ* synthesized to the waver [16]. Eleven to 16 probes (probe pairs) per gene are usually present on GeneChips, whereas on oligonucleotide and cDNA microarrays genes are represented by one or two probes.

Independent of the type of array used, massive data spanning a significant fraction of the presently known coding regions are generated by utilizing full genome chips, resulting in extended down-processing efforts. The tasks include quality control, normalization procedures and data complexity reduction. Subsequent analysis of array data spans explorative analysis and statistical testing, complemented by co-regulation analysis and identification of molecular pathways and protein interaction networks. A review on data processing and bioinformatics for identification of disease-associated proteins and involved functional networks is given in Perco *et al.* [17].

Due to heterogeneity of sample material and statistical considerations of analyzing a large number of genes based on a usually limited number of samples the false-positive rate of identified disease-associated genes and proteins is usually high. Consequently, independent experimental verification of results derived from differential gene expression experiments is inevitable. A review on general procedures of biomarker development covering identification and validation in the context of renal diseases was recently published by Hewitt *et al.* [18]. A scheme outlining our analysis flow concept is depicted in Fig. 1.

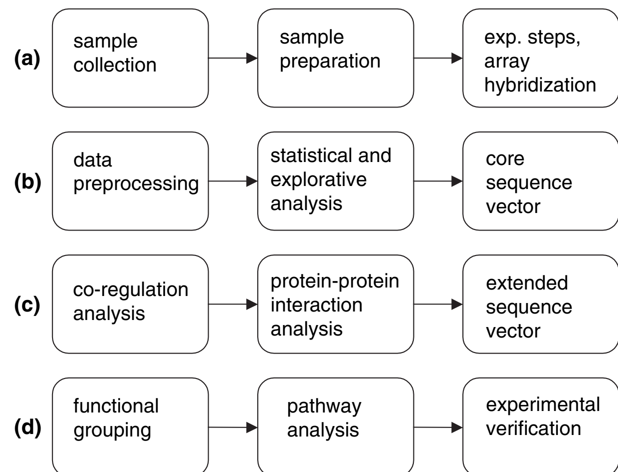

Figure 1 Differential gene expression analysis workflow. (a) Experimental steps toward generating the differential gene expression raw data. (b) Data preprocessing and statistical analysis for deriving a core sequence vector holding significantly differentially regulated genes. (c) Expansion of the core sequence vector via inclusion of sequences showing transcriptional co-regulation or interlinked proteins identified on the level of protein–protein interaction networks. (d) Analysis of identified functional groups and populated molecular pathways, complemented by prospective experimental verification.

The next sections outline gene expression studies focusing on ischemia reperfusion injury utilizing animal models as well as human sample materials.

Analysis of IRI – animal models

Array-based differential gene expression analysis targeting reperfusion injury has been performed in animal models [19–24] as well as in the human post-transplant situation.

Kieran *et al.* [23] analyzed the gene expression profiles in kidneys from NIH Swiss mice after ARF induction by clamping or folic acid treatment. The group reported 68 differentially regulated genes (44 upregulated) which were further analyzed concerning their expression profiles after lipoxin-mediated renoprotection.

Yoshida *et al.* [20] found 109 differentially regulated genes in an ischemia reperfusion injury ARF mouse model. A similar analysis was conducted in rats yielding 18 differentially regulated genes after ARF induction [19].

Supavekin and colleagues identified 91 upregulated and 156 downregulated genes after ARF induction in a mouse model using cDNA microarrays [21,22]. They concluded that apoptosis plays a major role in tubular damage following ischemia reperfusion injury.

Yuen *et al.* [24] used microarrays to identify early biomarkers for ischemia as well as for nephrotoxic ARF in rat ARF models. Sixty-two genes were differentially

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expressed after mercuric chloride administration, whereas 102 genes showed differential expression after ischemia reperfusion injury.

We have manually extracted the genes reported as differentially expressed in these papers. This meta-analysis of the six gene expression studies resulted in a gene list containing 457 entries representing 396 unique genes. Forty-one genes were reported in more than one publication and are listed in Table 1.

Six genes are reported four or five times to be upregulated shortly after ARF, among them the cyclin-dependent kinase inhibitor 1A (CDKN1A), also known as p21, claudin 7 (CLDN7) or galectin 3 (LGALS3). The epidermal growth factor (EGF) on the other hand is reported to be downregulated after ARF in two of five publications.

Genes upregulated in ARF are mainly involved in immunity and defense, ligand-mediated signaling, cell proliferation and differentiation, as well as in apoptotic processes. Four genes are members of the interleukin-signaling pathway, namely interleukin 6 (IL-6), CDKN1A, V-fos FBJ murine osteosarcoma viral oncogene homolog (FOS), as well as the TGFB-induced factor (TGIF).

Table 1. Genes reported as differentially regulated at least in two of five publications studying acute renal failure after reperfusion injury.

Symbol	Gene name	Up	Down
AKAP12	A kinase (PRKA) anchor protein (gravin) 12	4	0
CD68	CD68 antigen	3	0
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	3	0
CLDN7	Claudin 7	3	0
LGALS3	Lectin, galactoside-binding, soluble, 3 (galectin 3)	3	0
TUBB	Tubulin, beta	3	0
ACTA2	Actin, alpha 2, smooth muscle, aorta	2	0
ANXA2	Annexin A2	2	0
ANXA3	Annexin A3	2	0
CD14	CD14 molecule	2	0
CLU	Clusterin	2	0
COL18A1	Collagen, type XVIII, alpha 1	2	0
CXCL1	Chemokine (C-X-C motif) ligand 1	2	0
CYR61	Cysteine-rich, angiogenic inducer, 61	2	0
EGR1	Early growth response 1	2	0
FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog	2	0
FOSL1	FOS-like antigen 1	2	0
HAVCR1	Hepatitis A virus cellular receptor 1	2	0
HMOX1	Heme oxygenase (decycling) 1	2	0
HSPA1A	Heat shock 70 kDa protein 1A	2	0
IER2	Immediate early response 2	2	0
IL6	Interleukin 6 (interferon, beta 2)	2	0
LCN2	Lipocalin 2 (oncogene 24p3)	2	0
MT1A	Metallothionein 1A	2	0
PEA15	Phosphoprotein enriched in astrocytes 15	2	0
SPHK1	Sphingosine kinase 1	2	0
SRXN1	Sulfiredoxin 1 homolog (S. cerevisiae)	2	0
TGIF	TGFB-induced factor (TALE family homeobox)	2	0
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A	2	0
VCAM1	Vascular cell adhesion molecule 1	2	0
A2M	Alpha-2-macroglobulin	1	1
COL3A1	Collagen, type III, alpha 1	1	1
TNC	Tenascin C (hexabrachion)	1	1
LIFR	Leukemia inhibitory factor receptor	1	2
AFM	Afamin	0	2
AQP1	Aquaporin 1 (Colton blood group)	0	2
BCAT1	Branched chain aminotransferase 1, cytosolic	0	2
EGF	Epidermal growth factor (beta-urogastrone)	0	2
GAS2	Growth arrest-specific 2	0	2
MYO5A	Myosin VA (heavy polypeptide 12, myoxin)	0	2
SLC16A7	Solute carrier family 16, member 7	0	2

Given is the gene name, gene symbol, as well as the total number of papers reporting a gene as up- or downregulated.

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Analysis of IRI – human samples

We used cDNA microarrays to analyze the gene expression profiles of 'zero hour' renal allografts from living as well as from cadaveric donors. Clearly different expression profiles were identified for the two sample sources. We located a set of 132 genes and expressed sequence tags (ESTs) that clearly separated living from cadaveric donor organs [11]. In particular, members of the complement cascade and genes involved in immune response were activated in cadaveric, but not in living donor kidneys. These results are in line with findings of previous studies where key members of the inflammation cascade, namely adhesion molecules and mitochondrial controllers of apoptosis could be identified as being strongly differentially regulated in cadaveric donor organs compared with kidneys from live donors [25,26]. Kurian *et al.* [27] recently showed that even laparoscopic live donor nephrectomy causes inflammation in the graft compared to open nephrectomy.

A gene set consisting of 48 genes was identified in deceased donor kidney biopsies that separated organs with primary function from those with subsequent acute renal failure. All genes showed higher expression in ARF samples. The majority of these genes could be classified as members of signal transduction pathways and cell cycle regulation [11].

In another study, we utilized laser capture microdissection to isolate different compartments of the kidney, namely the glomeruli and the tubulointerstitial compartment, again studying live and cadaveric donor samples. We identified genes and ESTs that are differentially regulated in the different compartments of cadaveric donor organs and live donor transplant kidney biopsies obtained at time zero, reflecting miscellaneous events happening in the donor and during procurement [28]. The major finding was that genes counterbalancing oxidative injury are upregulated in the tubulointerstitial compartment of cadaveric kidneys when compared with living organs.

We are currently evaluating whether the gene expression profile of the donor organ at time point zero has a major effect on long-term allograft function. Preliminary results suggest that inflammatory processes reduce the long-term graft function. These findings are in line with the results obtained for acute renal failure after transplantation as reported by Hauser *et al.* [11].

Context analysis of reported marker proteins

To further investigate the functional roles of potential kidney biomarkers depicted in Table 1, as well as of the 48 genes reported by Hauser and colleagues as differentially regulated in transplant ARF, the total 89 unique genes were assigned to their molecular function and biological processes. Biological processes and molecular functions holding a significant number of genes were identified using the Gene Expression Data Analysis Tool of the PANTHER Classification System [29,30]. The number of genes in each category was compared with the PANTHER reference data set holding all 23 401 currently annotated human genes. The ratio of expected to observed frequency of genes assigned to certain ontology categories was compared by using a chi-squared test including Bonferroni correction to account for multiple testing.

Seventeen genes of the list of 89 candidate markers were assigned to the most significant group, namely immunity and defense. Other significantly enriched groups are cell cycle (12 genes), apoptosis (eight genes) or cell proliferation and differentiation (11). A detailed listing of all significant biological processes and molecular functions of the 89 potential biomarkers is given in Table 2.

Besides the functional annotation according to the PANTHER Classification System we used human protein–protein interaction (PPI) data to determine the connectivity of the 89 unique potential biomarkers on the level of cellular protein networks. High-quality protein interaction data from Online Predicted Human Interaction Database (OPHID) were used for the analysis [31]. Interaction networks were generated using the nearest neighbor expansion method as proposed by Chen *et al.* [32]. The level of aggregation and complexity of derived interaction networks were quantified by computing the Index of Aggregation (IA), given as the percentage of selected nodes in the largest subnetwork with respect to all selected nodes in the network. The IA of the biomarker network was compared to respective values of randomly

Table 2. Functional classification of the 89 proteins reported in the context of acute renal failure following our literature search as well as analysis of given differential gene expression data.

Biological process	Markers (89)	*P*-value
Immunity and defense	17	0.0001
Cell cycle	12	0.0037
Apoptosis	8	0.0185
Cell structure and motility	12	0.0224
Cell proliferation and differentiation	11	0.0357
Molecular function	Markers (89)	*P*-value
Transfer/carrier protein	7	0.0047

Presented are the biological process/molecular function, the number of markers belonging to a particular process/function, as well as the significance of the enrichment of a given biological process/function.

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generated protein lists. Forty-three of the 65 proteins which actually have interaction entries in the OPHID database were connected in a single subgraph after the one neighbor expansion method. The resulting IA of 0.66 is more than two standard deviations above the expected IA for randomly generated networks of equivalent size. Figure 2 shows the IA of the given marker list, as well as a representation of the connectivity of identified protein interaction networks.

Based on this analysis, candidate marker proteins presently identified are significantly interlinked on the level of protein–protein interactions. These findings point towards a functional link, potentially even-concerted molecular processes, of the candidate biomarkers presently associated with ARF.

Molecular biomarkers of ischemia reperfusion injury

Molecular biomarkers, derived from deductive as well as explorative approaches, are partially entering first verification studies aimed at demonstrating their potential in early diagnosis or even risk assessment of ARF. Based on a literature review, we have identified 12 proteins reported as potential biomarker in the context of acute renal failure as listed in Table 3.

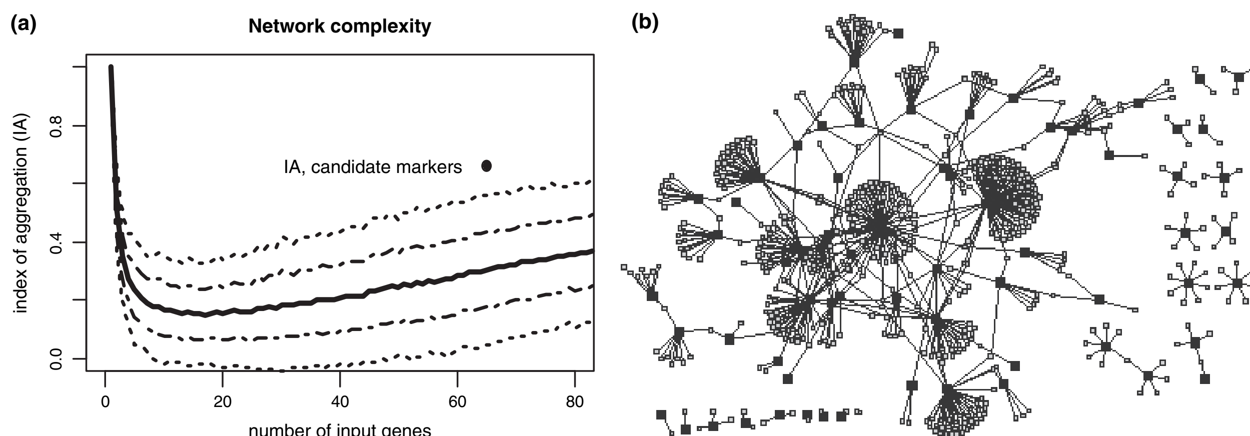

Figure 2 Protein–protein interaction networks based on given biomarker candidate proteins. (a) The index of aggregation (IA) (*y*-axis) depending on the number of proteins used for constructing protein interaction networks (*x*-axis). The IA of the given list of marker candidates is significantly above the IA of randomly selected lists. (b) Protein interaction networks as given by the candidate marker list of 89 proteins. Black boxes indicate proteins given in the marker candidate list; grey boxes indicate proteins identified by the next nearest neighbor expansion.

Table 3. Protein markers reported in the context of reperfusion injury/acute renal failure.

Gene name	Gene symbol	Source	References
Candidate markers, acute renal failure			
Actin, alpha 2, smooth muscle, aorta	ACTA2	Renal transplant patients	Badid *et al.* [33]
Uromodulin	UMOD	Patients with renal disease; renal transplant patients	Lynn and Marshall [37]; Zimmerhackl [36]
Lectin, galactoside-binding, soluble 3	LGALS3	Rat ARF model	Nishiyama *et al.* [39]
Spermidine/spermine N1-acetyltransferase	SAT	Rat ARF model	Zahedi *et al.* [41]
Hepatitis A virus cellular receptor 1	HAVCR1	Rat ARF model	Ichimura *et al.* [43]; Hong *et al.* [44]; Vaidya *et al.* [45]
Chemokine (C-X-C motif) ligand 1	CXCL1	Mouse ARF model	Molls *et al.* [46]
Annexin A2	ANXA2	Mouse ARF model	Cheng *et al.* [47]
S100 calcium binding protein A6	S100A6	Mouse ARF model	Cheng *et al.* [47]
Cysteine-rich angiogenic inducer 61	CYR61	Mouse and rat ARF model	Muramatsu *et al.* [48]
S100 calcium binding protein beta	S100B	Rat ARF model	Pelinka *et al.* [50]
Alpha-1-microglobulin	AMBP	Patients with acute tubular necrosis	Herget-Rosenthal *et al.* [53]
Lipocalin 2	LCN2	Mouse ARF model; children undergoing cardiopulmonary bypass	Mishra *et al.* [55,56]

Presented are the gene name, the gene symbol, as well as the experimental setup for identifying the protein markers (source), as well as major references.

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Most of the candidate markers have been discovered and analyzed utilizing animal models, complemented by human post-transplant ARF study cases. Badid *et al.* [33] reported a correlation between smooth muscle actin alpha (ACTA2) and the annual loss in glomerular filtration rate (GFR) in renal allografts, thus proposing ACTA2 as a new biomarker to predict renal function after transplantation. The same group was able to demonstrate the prognostic potential of ACTA2 in membranous nephropathies [34]. A review on myofibroblasts expressing ACTA2 in the context of various diseases was provided by Powell *et al.* [35].

The downregulation of uromodulin (UMOD, also known as Tamm–Horsfall protein) in the context of renal transplantation and acute renal failure was demonstrated by several groups [36,37]. A review on mutations of the uromodulin gene and its role in kidney disease was recently published by Scolari *et al.* [38].

Nishiyama *et al.* could demonstrate the upregulation of Galectin-3 (LGALS3) expression after ischemic as well as toxic acute renal failure in a rat model [39]. A high correlation between serum creatinine values and galectin-3 mRNA expression was shown suggesting an involvement of LGALS3 in the pathophysiology of ARF. Galectin-3 is embedded in protein interaction networks involved in cell to cell adhesion including collagens, laminins and elastin, as well as anti-apoptotic proteins like BCL2 [40].

A protein upregulated after renal ischemia reperfusion injury is spermidine/spermine N1-acetyltransferase (SAT), as demonstrated by Zahedi *et al.* [41] in a rat model using Northern as well as Western blots. This finding was supported by Barone *et al.* [42] obtaining similar results for SAT in a mouse model of ischemia reperfusion injury. Enhanced SAT expression reduces the amount of intracellular polyamines, whereas toxic metabolites are formed (putrescine and H_2O_2). These processes lead to growth arrest and cellular damage. The overexpression of the cyclin-dependent kinase inhibitor 1A (CDKN1A), also known as p21, after SAT induction is worth mentioning as CDKN1A is also reported as associated with renal failure [42].

The hepatitis A virus cellular receptor 1 (HAVCR1), also known as kidney injury molecule-1 (Kim-1), was reported to be highly upregulated in kidney tissue in response to various nephrotoxins suggesting a possible role of Kim-1 as a biomarker for renal injury [43,44]. Vaidya *et al.* [45] used a sandwich ELISA to detect the amount of urinary Kim-1 in order to assess early kidney injury.

Molls *et al.* [46] detected an increase in keratinocyte-derived chemokine (CXCL1) after ischemia reperfusion injury in a mouse model using protein arrays. Upregulated CXCL1 levels in serum and urine could be detected earlier than a significant rise in serum creatinine levels. Stimuli for CXCL1 production are, among others, the inflammatory proteins IL1 and IL4, the tumor necrosis factor alpha (TNF) and interferon gamma (IFNG). These proteins are frequently present at sites of tissue damage [46].

Two other proteins, namely annexin a2 (ANXA2) and S100 calcium binding protein A6 (S100A6), showed elevated levels in RT-PCR experiments as well as immunohistochemistry after acute renal failure. These two proteins are probably involved in regulation of renal cell proliferation and recovery processes of tubular cells in tubular necrosis, according to Cheng *et al.* [47].

Muramatsu *et al.* [48] reported elevated levels of serum and urinary cysteine-rich angiogenic inducer 61 (CYR61) in rat and mouse models of ARF. CYR61 is a member of the CCN family and is involved in cell adhesion, extracellular matrix production and cell growth due to the ability to bind cell surface integrins [49].

In a similar rat model of ischemia, Pelinka *et al.* [50] were able show the upregulation of S100 calcium binding protein beta (S100B) after the onset of reperfusion in a rat ARF model. S100B is, in addition, a marker of brain damage and was postulated as prognostic marker in melanoma patients [51,52].

Herget-Rosenthal *et al.* identified alpha-1-microglobulin (AMBP) as potential marker to predict the unfavorable outcome of renal replacement therapy in patients showing proteinuria [53]. AMBP is a member of the lipocalin superfamily [54].

Another member of the lipocalin superfamily, and presently one of the most promising biomarkers for the early detection of acute renal failure is the neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin 2 (LCN2) [55]. Mishra *et al.* [56] used Western blot and ELISA techniques to measure NGAL expression both in serum and urine. A correlation between NGAL expression and acute renal injury was observed. Sensitivity and specificity values for urine NGAL concentrations reached values of 1.00 and 0.98, respectively, for the patient cohort under analysis. The role of LCN2 in iron transport and formation of renal epithelium was reviewed by Yang *et al.* [57].

The list of genes presently associated with acute renal failure shows elements mainly involved in the biological processes of signal transduction, cell cycle regulation, cell structure or immunity and defense.

Conclusion and outlook

Following verification procedures of marker proteins associated with ARF, as well as based on analysis of associated functional pathways a set of diagnostic and therapeutic options has been derived.

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For diagnostics substantial further prospective testing has to be performed. For example, LCN2 has already demonstrated excellent sensitivity and specificity for early identification of ARF in a defined patient cohort. General applicability still has to be shown.

Presently outlined therapeutic options are manifold. For example, neutrophil infiltration in postischemic kidneys is thought to be associated with development of ARF. Adhesion molecules responsible for neutrophil recruitment are therefore a possible target to protect the kidney from ischemia/reperfusion-induced ARF [58]. Caspase-1 inhibitor II admitted prior to renal ischemia reperfusion injury was shown to protect the kidney against renal dysfunction and oxidative stress in a rat model. The inhibitor reduced both the ICAM-1 expression and the MPO activity, probably leading to a reduced neutrophil infiltration [59].

P-selectin was described as prerequisite for developing postischemic ARF following results of animal studies. Selective blocking of platelet P-selectin could show protective effects [60].

CD55 and CD59 are regulatory proteins of the membrane complement system, and data suggest that these proteins play a role in preventing renal ischemia reperfusion injury. Deletion of both proteins led to increased renal ischemia reperfusion, whereas CD59 deficiency alone did not show an increased sensitivity in the context of reperfusion injury. It is suggested that CD55 is a key protein for the initiation of the complement activation following renal ischemia reperfusion, and that it acts synergistically with CD59 to inhibit complement activated renal injury [61].

Cervastatin treatment for 3 days showed significant effects on ischemia-induced ARF in a rat model, as the decrease of renal function was minimized. Furthermore it was observed, that especially the S3 segment of proximal tubules showed a reduced damage.

Reduced levels of ICAM-1, iNOS or ERK1/2 activation suggest anti-inflammatory effects of statin [62].

Based on our findings on increased expression activity in the context of the functional groups immune response and inflammation along cadaveric donor organs we have started a multicenter, randomized placebo controlled trial performing immunosuppressive treatment of the cadaveric organ donor before graft harvesting. Previous trials on donor pretreatment were carried out in the late 1970s and early 1980s, but severe problems in the design and sample size of all three randomized studies performed so far preclude a clear cut answer [63–65].

Summing up, a selected number of biomarkers of ischemia reperfusion injury has been identified utilizing animal models and human biopsy samples, and selected candidates have undergone experimental validation. Furthermore, novel therapy approaches are under consideration utilizing knowledge on functional processes and pathways addressed by the identified biomarkers.

Combining this preclinical knowledge with full genome gene expression profiles clearly has the potential of deepening our understanding of concerted activities on a cell-wide level, providing the ground for tackling ischemia reperfusion injury in the clinical context.

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