Acute kidney injury transcriptomics unveils a relationship between inflammation and ageing

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ABSTRACT

There are no pathophysiolgical therapeutic approaches to acute kidney injury (AKI) and the mortality remains high. In addition chronic kidney disease (CKD) predisposes to AKI and AKI contributes to progression of CKD. Recently a transcriptomics approach unveiled a relationship between AKI, inflammation and the regulation of ageing. A transcriptomics analysis of experimental AKI revealed increased kidney expression of Fn14 and transmembrane chemokine CXCL16, as well as a decreased expression of the kidney-secreted anti-ageing hormone Klotho. Fn14 is the receptor for tumor necrosis factor-like weak inducer of apoptosis (TWEAK), a member of the TNF superfamily. In AKI kidneys there was a positive correlation between Fn14 and CXCL16 mRNA expression and an inverse correlation between Fn14 and Klotho mRNA. Tubular cells were the site of Fn14, CXCL16 and Klotho expression in vivo. Research on the relationships between these three molecules disclosed that TWEAK activation of Fn14 promoted inflammation through secretion of chemokines such as CXL16 in tubular cells in culture and in vivo. Furthermore, TWEAK activation of Fn14 decreased expression of Klotho mRNA and protein in culture and in vivo. Interestingly, both TWEAK activation of CXCL16 mRNA transcription and suppression of Klotho mRNA transcription were mediated by the NFκB transcription factor. In conclusion, TWEAK engagement of Fn14 is a central event promoting NFκB-mediated activation of inflammation pathways and suppression of anti-inflammatory/anti-ageing pathways. This information may influence future therapeutic approaches to AKI and inflammation/aging.

Keywords: Acute kidney injury. Aging. Chronic kidney disease. Inflammation. Klotho. TWEAK.

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La transcriptómica del fracaso renal agudo revela una relación entre inflamación y envejecimiento

RESUMEN

No existen estrategias terapéuticas y fisiopatológicas para el fracaso renal agudo (FRA), por lo que los niveles de mortalidad continúan siendo elevados. Además, la enfermedad renal crónica (ERC) predispone a sufrir FRA y el FRA, a su vez, contribuye a que la ERC avance. Recientemente, una estrategia transcriptómica reveló una relación entre el FRA, la inflamación y la regulación del envejecimiento. Un análisis transcriptómico de modelos experimentales de FRA reveló un aumento de la expresión renal de Fn14 y la quimiocina transmembrana CXCL16, así como un descenso en la expresión de la hormona Klotho antienvejecimiento secretada por el riñón. Fn14 es el receptor de la citoquina tumor necrosis factor-like weak inducer of apoptosis *(TWEAK), miembro de la superfamilia de factor de necrosis tumoral. En los riñones con FRA, existía una correlación positiva entre Fn14 y la expresión de ARNm de CXCL16 y una correlación inversa entre Fn14 y el ARNm de Klotho. El lugar donde se da la expresión* in vivo *de Fn14, CXCL16 y Klotho es las células tubulares. La investigación en las relaciones entre estas tres moléculas reveló que la activación de Fn14 por TWEAK provocó la inflamación mediante la secreción de quimiocinas como la CXCL16 en células tubulares, tanto en cultivo como* in vivo. *Además, la activación de Fn14 por TWEAK disminuyó la expresión de ARNm de Klotho y de proteína, en cultivo e* in vivo. *Curiosamente, tanto la activación TWEAK de la trascripción de ARNm de CXCL16 y la supresión de la trascripción de ARNm de Klotho estuvieron mediadas por el factor de transcripción NF-κB. Como conclusión, la unión de TWEAK y Fn14 es un elemento clave en promover de la activación mediada por NF-κB de las vías de inflamación y en la supresión de las vías antiinflamatorias y antienvejecimiento. Esta información puede influir en las futuras estrategias terapéuticas para el FRA y la inflamación/envejecimiento.*

Palabras clave: *Fracaso renal agudo. Envejecimiento. Enfermedad renal crónica. Inflamación. Klotho. TWEAK.*

ACUTE KIDNEY INJURY AND CHRONIC KIDNEY DISEASE

Acute kidney injury (AKI) is a syndrome characterized by tubular injury and a sudden drop in glomerular filtration. Our

current understanding of the pathophysiology of AKI is incomplete and this accounts for the lack of specific therapy. One key feature that has emerged in recent years is the close relationship between AKI and chronic kidney disease (CKD).[1] Thus, CKD is the main risk factor for AKI and AKI contributes to progression of CKD. This suggests that AKI and CKD share pathogenic factors: from a pathogenic point of view CKD may be considered a low level, persistent AKI. Since pathogenic events are magnified in AKI and AKI has a shorter time course, AKI has advantages as a model for the identification and assessment of pathogenic factors. In this regard, proposed biomarkers of AKI are also altered in CKD, including Klotho.[2,3]

TRANSCRIPTOMICS

High throughput techniques such as transcriptomics and proteomics, may help identify novel potential pathogenic factors, therapeutic targets and biomarkers in a non-biased way.[4-6] Transcriptomics is a high throughput technique that allows the identification of thousands of differentially expressed candidate genes. Such patterns of expression may themselves be used for diagnostic or prognostic purposes. Bioinformatics and biostatistics tools allow to manage thousands of genes simultaneous and help to prioritize molecules for further confirmatory studies. Novel therapeutic targets may be uncovered. We recently used a transcriptomic approach to identify new genes involved in AKI that could serve as biomarkers or therapeutic targets.[7,8] This approach has successfully identified new players in diabetic nephropathy such as the lethal cytokine TRAIL; the MIF receptor CD74 and the intracellular lethal protein BASP1.[9-12]

TWEAK AND FN14

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK, Apo3L, TNFSF12) is a member of the tumor necrosis factor superfamily (TNFSF).[13-15] Other members of the family include TNF and Fas ligand, both of which play a key role in kidney injury.[16-18] TNFSF ligands bind to one or more members of the TNF receptor superfamily (TNFRSF).[19,20]

The human TWEAK gene encodes a type II transmembrane glycoprotein. The TWEAK C-terminal extracellular domain contains the TNF homology domain that mediates self-trimerization and receptor-binding.[13] The N-terminal intracellular domain contains several nuclear localization sequences (NLS)[13,21-23] and a furin recognition site, suggesting that TWEAK can be cleaved.[24] Most cells can express full-length membrane-anchored TWEAK (mTWEAK) and soluble TWEAK (sTWEAK).[24,25] sTWEAK is formed by proteolysis of membrane TWEAK.[13,25,26]

Both sTWEAK and mTWEAK bind and activate fibroblast growth factor-inducible-14 (Fn14, TWEAK receptor, TNFRSF12A, CD266).[24,27-29] Fn14 was initially described in fibroblasts as a growth factor-regulated early response gene.[30] Fn14 is a type I transmembrane protein that when mature has 102-aa. Fn14 is the smallest member of TNFRSF. The intracellular Fn14 domain contains TNFR-associated factor (TRAF)-binding sites which activate signal cascade. Unlike TNF or Fas, Fn14 does not contain a death domain (DD).[31] In addition, CD163 binds TWEAK and is thought to be a TWEAK scavenger receptor, since TWEAK-induced signaling through CD163 was not observed.[32,33]

TWEAK has multiple functions with potential physiopathological relevance for kidney injury that depend on the microenvironment, the cell type and the cell state of activation. TWEAK can regulate cell proliferation, cell death, cell migration, cell differentiation, tissue regeneration, neoangiogenesis and inflammation.[34-43] TWEAK contributes to tissue injury in the central nervous system, liver, gut, the vasculature, skeletal muscle, heart and kidney.[40,44-49]

In the kidney TWEAK actions have been extensively studied in tubular epithelium. TWEAK induces proliferation in non-stressed renal tubular cells[50] and apoptosis in tubular cells stressed by an inflammatory milieu.[14,51] Furthermore TWEAK activates both canonical and non-canonical NFκB transcription factor signaling[14,52-54] Through these actions TWEAK promotes tubular injury in ischemic or toxic AKI[54,55] and kidney hyperplasia following unilateral nephrectomy.[56] Furthermore, TWEAK contributes to vascular injury and in CKD patients soluble TWEAK behaves as a biomarker of outcome, especially when interpreted in the context of systemic inflammation.[57-61] A recent transcriptomics analysis of kidney tissue in AKI confirmed highly upregulated levels of Fn14 mRNA (Figure 1). Regulation of the TWEAK/Fn14 system often takes places through upregulation of receptor expression and, thus, of cell sensitivity to TWEAK actions.

CXCL16

Chemokines are small cytokines formerly known as intercrines[62] that in the kidney tubulointerstitium may be expressed by both tubular cells and fibroblasts.[63] Chemokines promote leukocyte trafficking, growth and activation in inflammatory sites.[64] Chemokines promote kidney tubulointerstitial inflammation.[65,66] Leukocytes recruited by chemokines have a key role in kidney tubulointerstitial tissue injury during AKI and CKD.[67,68] Several chemokines were upregulated in the transcriptome of murine AKI.[8] Some of them, such as MCP-1 and Rantes, had already been studied.[54] These chemokines share with most chemokines their release as soluble mediators.[65] CX3CL1 (fractalkine) and CXCL16 (SR-PSOX) were also found

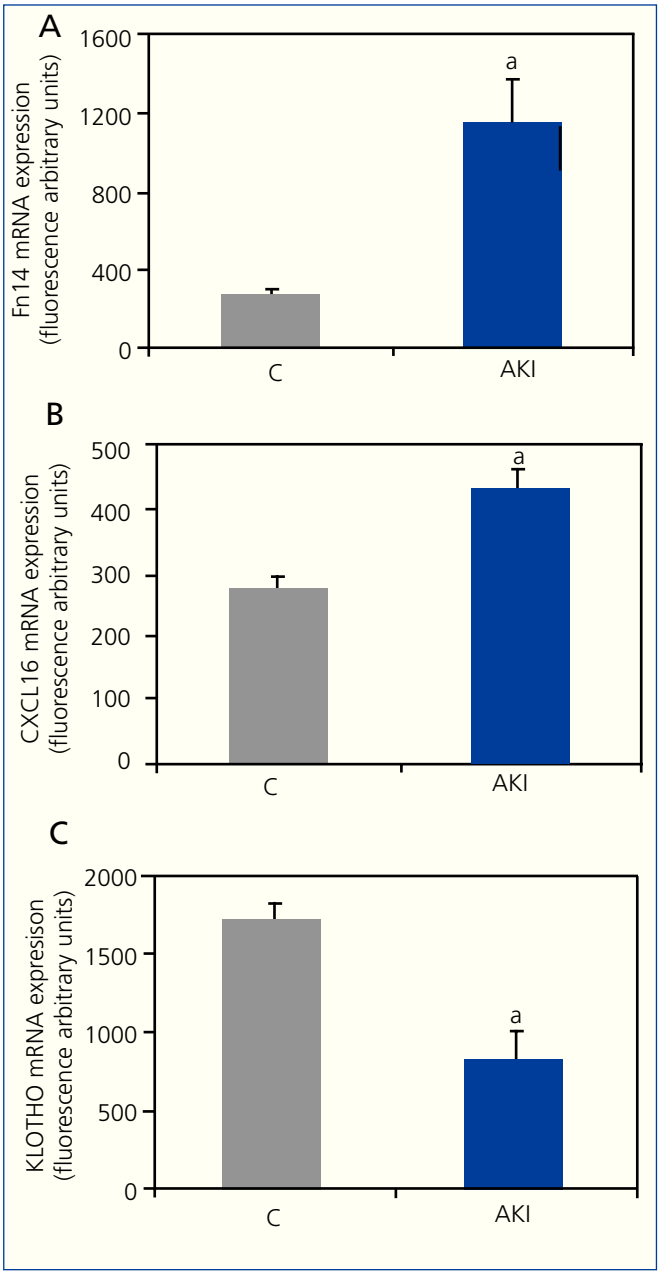

Figure 1. Gene expression for representative mediators of inflammation and ageing in experimental acute kidney injury (AKI): Transcriptomics results of kidney tissue.
A) Fn14 mRNA. B) CXCL16 mRNA. C) KLOTHO mRNA.
[a] p<.005 vs vehicle-injected controls (C). Data expressed as mean (SEM).

upregulated in the murine AKI the transcriptome.[8] CX3CL1 and CXCL16 are the only two known membrane-anchored chemokines.[69] CX3CL1 and CXCL16 are synthesized as transmembrane molecules and, as such, have specific functions that may go beyond their chemokines role. In addition, they can be cleaved from the cell surface to release a soluble chemoattractant that behaves as a classical chemokine.[69] Fractalkine has been extensively studied in the context of kidney disease.[70] However, much less was known about CXCL16 and kidney injury. Furthermore, CXCL16 expression correlated more closely than CX3CL1 with Fn14 expression.[8] In addition, there is evidence that in humans urinary TWEAK and CXCL16 may be a potential diagnostic biomarkers of kidney diseases such as lupus nephritis.[71,72] TWEAK is known to regulate the expression of several chemokines. These peculiarities made a complete understanding of the relationship between TWEAK and CXCL16 regulation in kidney cells of particular interest.

CXCL16 was identified by different groups as a ligand for the CXC-chemokine receptor CXCR6[73] and as a scavenger receptor for phosphatidylserine and oxidized low density lipoprotein (oxLDL) and therefore was also termed SR-PSOX.[74] Full length CXCL16 consists of an extracellular N-terminal chemokine domain, a glycosylated mucin-like stalk, a transmembrane-spanning region and a short cytoplasmic tail.[73] Like CX3CL1, CXCL16 potentially functions as both a soluble chemokine and a membrane-bound adhesion molecule.[75-78] CXCL16 regulated leukocyte chemotaxis, T cell recruitment and cell proliferation.[79-87]

In the kidney, CXCL16 is constitutively expressed in human mesangial cells, podocytes and tubular cells.[79,86,87] There is evidence for differential regulation of CXCL16 expression in glomeruli or different tubular segments and in tubular injury of diverse etiology. CXCL16 expression is increased in various animal models of kidney injury and human nephropathies.[72,79,86-89]

Glomerular CXCL16 expression is increased in human membranous nephropathy.[87] Glomerular and tubular CXCL16 was also increased in lupus mice and in anti-GBM nephritis.[72,88,89] Functional studies suggest that CXCL16 promotes progression of damage in experimental glomerulonephritis.[88] CXCL16 blockade significantly decreased monocyte/macrophage infiltration and glomerular and tubular injury.[88,89] In this regard, besides effects on leukocytes, CXCL16 has direct actions on glomerular cells. Podocyte CXCL16 may regulate the uptake of oxLDL,[87] while mesangial cell CXCL16 promotes mesangial cell migration and proliferation.[79]

In human allograft AKI, CXCL16 expression was increased focally in the apical side of tubules.[86] By contrast, a low tubular CXCL16 expression was observed in interstitial rejection that was attributed to increases CXCL16 shedding. Thus, remnant CXCL16 was located to the basolateral membrane and surrounded by T cell infiltrates. In experimental toxic AKI, both prominent apical and basolateral CXCL16 expression were noted.[8] Thus, other tubular cells, the interstitium and the tubular lumen were exposed to CXCL16 derived from tubular cells. Interestingly, both patterns did not overlap in many tubules.

No in vivo functional studies of CXCL16 targeting in tubulointerstitial kidney disease have been reported. In cell culture CXCL16 did not induce murine tubular epithelial cell proliferation or apoptosis, either alone or in combination with TWEAK.[8] However, CXCL16 had a proinflammatory effect and increased TWEAK-induced gene expression of ICAM-1, MCP-1 and RANTES. In this regard, tubular cells expressed the CXCR6 receptor.[8]

In cultured glomerular cells CXCL16 is upregulated by TNF-α and IFN-γ.[79,86,87] IFN-γ increased CXCL16 expression in cultured primary thick ascending limb cells and early distal tubular cells.[86] TWEAK is a novel regulator of CXCL16 expression in tubular epithelial cells.[8] TWEAK promoted CXCL16 expression through the canonical NFκB pathway in cultured tubular cells.[8] Moreover, TWEAK increased renal CXCL16 expression and interstitial CD3 positive lymphocytes. Since neutralization of TWEAK decreased CXCL16 and CD3 lymphocyte infiltration in experimental AKI, TWEAK-induced CXCL16 expression may contribute to T cell recruitment and collaborate with TWEAK in promoting inflammation.

KLOTHO

Klotho is a protein with anti-aging properties which is highly expressed in tubular renal cells.[90,91] Klotho is a single-pass transmembrane protein. The extracellular domain of Klotho may be proteolytically processed by ADAM10/17 and secreted. In addition, alternative splicing may give rise to a soluble secreted isoform.[92] Trasmembrane Klotho binds to multiple fibroblast growth factor (FGF) receptors conferring them specific and high affinity for FGF23. FGF23 is a bone-derived hormone that regulates phosphate homeostasis and vitamin D metabolism. Thus, the main known function of Klotho is regulation of phosphate metabolism and evidence from mice in which phosphate was manipulated genetically or through diet suggests that aberrant phosphate homeostasis is a key contributor to the accelerated aging syndrome of Klotho -/- mice.[93] Klotho also protects cells and tissues from oxidative stress and has anti-inflammatory properties through modulation of NFκB signaling.[94]

Klotho is downregulated during kidney diseases, such as long-term hypertension, diabetes mellitus, CKD,[95] and in experimental AKI induced by ischemia-reperfusion or a folic acid overdose.[7,96] In addition kidney Klotho was decreased in the course of systemic inflammation caused by inflammatory bowel disease and a neutralizing anti-TNF antibody attenuated bowel inflammation and reversed the repression of kidney Klotho expression.[97] Consistent with these data, Klotho was downregulated in the transcriptome of murine AKI and Klotho expression was inversely correlated with Fn14 expression, suggesting that TWEAK, like TNF, may regulate Klotho expression. The reduction of kidney Klotho during nephrotoxic AKI persisted beyond recovery of renal function and was associated with decreased circulating Klotho. The persistent decrease in Klotho might be related to the increased mortality of AKI patients following recovery from AKI. Since Klotho may be nephroprotective,[96,98-100] the persistent decrease in Klotho might also predispose to progression of CKD. However, these hypotheses await formal confirmation.

In nephrotoxic AKI, Klotho expression and renal function were preserved by TWEAK targeting thus identifying a potential regulator of Klotho expression in cultured cells.[7] Indeed, in cultured tubular cells of proximal origin TWEAK and TNF promoted the NFκB-dependent downregulation of Klotho expression.[7] TWEAK and TNF activate the canonical pathway for NFκB activation, but only TWEAK activates the non-canonical pathway.[14,53] The reported downregulation of Klotho by TNF[7,97] and the time course of Klotho mRNA downregulation, that is already observed at 3h, suggest activation of the canonical NFκB pathway. Indeed, RelA was necessary for TWEAK- and TNF-induced Klotho repression. For the first time it was observed TWEAK downregulates NFκB-mediated gene expression. Regulation of NFκB activation function is controlled through different mechanism, such as interaction of the p65/RelA subunit with histone deacetylase (HDAC) corepresor proteins.[101-103] In this regard, HDAC inhibitors prevented repression of Klotho induced by TWEAK or TNF. In addition, recruitment of NFkB to chromatin is regulated in a promoter-specific manner. TWEAK induced histone H3 and H4 deacetylation at the murine Klotho promoter in renal tubular cells.

INTERACTION BETWEEN INFLAMMATION AND AGEING: NFκB

From the above mentioned studies the NFκB emerges as a family of pleiotropic transcription factors with a key role at the interface between inflammation and ageing.[52,104-107] This notion had been advanced before by proponents of the inflamma-aging concept.[108] The term inflamm-aging has been used to describe the age-related increase in the systemic pro-inflammatory status of humans.[109]

A wide range of stimuli relevant to tissue injury activate NFκB, including cytokines, growth factors, immune mediators, proteinuria and genotoxic or mechanical stretch.[110,111] Activation of NFκB can proceed through classical/canonical, alternative/non-canonical NFκB and hybrid pathways.[104,106,112] Classical NFκB activation is usually a rapid and transient response to a wide range of stimuli. Under basal conditions NFκB is inactive in the cytosol because it is bound to inhibitory IκB proteins. Activating stimuli activate the inhibitor of κB kinases (IKK), which phosphorylate IκBs, marking them for degradation by the proteasome. Degradation of IκB releases and activates

NFκB dimers, such as those containing RelA. RelA containing dimers then migrate to the nucleus where they bind to κB DNA sequences in promoters and enhancers of target genes. In general canonical NFκB promote the transcription and expression of proinflammatory genes, as observed for CXCL16 in TWEAK-stimulated tubular cells. There are several negative feed-back mechanisms. Thus, suppressors of cytokine signaling (SOCS)-1 promotes the ubiquitination and proteasomal degradation of RelA-containing dimers, thus quenching the NFκB response.[113] The SOCS1 overexpression decreases inflammation in experimental DN.[114]

As a result of NFκB integration of stimulus information it may both induce or repress individual gene transcription.[115] However, the fact that NFκB can function as a repressor of gene expression is less well-known. Gene expression repression by NFκB may suppress the inflammatory response by recruiting inhibitory components of the NFκB system. Thus, antiinflammatory cytokines, such as IL-10 promote synthesis of nuclear located atypical IκB proteins B-cell lymphoma 3 (BCL-3), IκBζ and IκBNS, which bind to DNA-bound NFκB dimers and may repress transcription of inflammatory genes.[113] In addition repression of gene expression by NFκB has been implicated in sepsis-induced downregulation of kidney aquaporin/V2 receptor and may have a role in resolution of inflammation.[116,117] However, classical NFκB dimers containing RelA may also downregulate Klotho mRNA and Klotho-dependent anti-inflammatory and ageing pathways, as observed for TWEAK and TNF, and, thus, promote further injury in and outside the kidney.

CONCLUSIONS

In summary, transcriptomics of AKI tissue has identified TWEAK as a novel regulator of CXCL16 expression in renal tubular cells through activation of the RelA NFκB transcription factor. In addition, TWEAK, like TNFα, downregulated Klotho in renal tubular cells through a similar NFκB RelA-dependent mechanism. Since Klotho has anti-ageing and anti-inflammatory properties, these findings may have therapeutic implications in kidney injury and also for inflammation-associated premature aging. Thus targeting either TWEAK, through neutralizing anti-TWEAK antibodies currently undergoing clinical trials in lupus nephritis, or targeting NFκB, may potentially limit inflammation and the adverse consequences of inflammation on ageing.

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Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

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KEY CONCEPTS

1. Tissue transcriptomics allows the non-biased analysis of gene expression and identification of potential novel therapeutic targets in tissue injury.
2. Acute kidney injury transcriptomics identified the simultaneous upregulation of inflammatory genes such as the TWEAK receptor FN14 and chemokines like CXCL16 and the downregulation of anti-inflammatory/anti-ageing genes such as Klotho.
3. TWEAK stimulation of tubular cells in culture reproduced the findings in AKI.
4. The transcription factor NFκB appears to be the key to both upregulation of proinflammatory genes and downregulation of Klotho in response to TWEAK.
5. Thus either targeting TWEAK, through neutralizing anti-TWEAK antibodies currently undergoing clinical trials in lupus nephritis, or targeting NFκB, may potentially limit inflammation and the adverse consequences of inflammation on ageing.

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