In search of early events in the development of chronic kidney disease: the emerging role for lipocalin-2/NGAL*

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Summary of key findings

In the October 2010 issue of the Journal of Clinical Investigation, Viau et al. [1] provided primary evidence for lipocalin-2 ([Lcn2; also known as neutrophil gelatinase-associated lipocalin ([NGAL]), 24p3 protein, α1-microglobulin-related protein, or uterocalin]) as a central effector of progressive renal tissue damage upon acute kidney injury. Their studies are based on two experimental mouse strains which differed profoundly in their responses to 75% nephrectomy: whereas FVB/N mice develop severe renal lesions resembling features of human chronic kidney disease (CKD), B6D2F1 mice are protected from early deterioration and instead exhibit compensatory alterations only. Post-surgical microarray analyses of the remnant renal tissues unravelled Lcn2 as the most markedly up-regulated gene in the FVB/N mice when compared to the B6D2F1 strain. Moreover, renal expression levels and urinary excretion of Lcn2 highly reflected the degree of tubular damage in the FVB/N mice, quite similar to humans with various forms of chronic kidney disease (CKD). Introduction of a homozygous disruption of the Lcn2 gene (i) largely abolished tubular cell proliferation, (ii) prevented the development of chronic renal lesions and (iii) preserved kidney function in FVB/N mice. The authors also identified Lcn2 as a downstream mediator following epidermal growth factor receptor (EGFR) activation. Genetically engineered mice with impaired EGF signalling did not up-regulate their Lcn2 levels and developed less severe renal damage in the remaining tissue after nephrectomy. Furthermore, EGFR activation mediated protein stabilization of the hypoxia-inducible factor (HIF)-1α, which accounted for increased Lcn2 expression.

Review of the field

Acute kidney injury (AKI) is diagnosed in 5% of hospitalized patients and up to 50% of all intensive care unit (ICU) patients [2]. Despite major advances in the treatment of AKI, increased long-term morbidity is a frequent complication of renal injury, and the 5-year survival rate after AKI is projected to be between 65 and 70% only [3]. Early diagnosis of AKI—even before a significant loss of renal function occurs—still poses a major challenge for clinicians since traditional and easily accessible renal biomarkers such as serum creatinine and blood urea nitrogen lack the capacity, sensitivity and specificity to detect renal impairment at early stages. Since, AKI is considered one of the most prominent risk factors for the development of end-stage renal disease as well as non-renal complications [4], there is a major interest in the identification of new markers that reliably reflect the degree of renal dysfunction early on after kidney injury.

In the last decade, several genomic, transcriptomic and proteomic approaches have pointed to Lcn2 as a promising biomarker for AKI [5–7]. These data were not only derived from animal models of ischaemic or nephrotoxic kidney injury, but have also been evaluated in human AKI in various clinical situations. In a recent meta-analysis reviewing more than >2,500 patients, Haase et al. [8] concluded that urinary as well as plasma Lcn2 serves as a useful diagnostic and prognostic marker for AKI.

Lcn2 (also referred to as NGAL) is a small secreted 25-kDa monomer of the lipocalin superfamily, which shares a highly conserved structure of an 8-stranded antiparallel β barrel (Figure 1) and comprises carrier proteins transporting iron chelators, retinoids and other hydrophobic molecules [9]. Several studies indicate that Lcn2 is induced under conditions like intoxication, inflammation and other forms of cellular stress. Furthermore, it plays a role in regulating haematopoiesis and has recently been implicated in epithelial-to-mesenchymal (EMT) transition during breast cancer progression [10]. Although this gene is widely expressed in many tissues and linked to many different functions, most data currently focus on its pathophysiology in renal diseases.

The study by Viau et al. confirms the significant up-regulation of Lcn2 after experimental nephron reduction...
using an unbiased genomic approach. More importantly, the authors demonstrate that Lcn2 is not only a biomarker but also a major effector mechanism of renal damage. In particular, they unravel functional propensities of Lcn2 as a downstream mediator of EGFR receptor activation and HIF-1α signalling and thereby the underlying molecular pathways that lead to increased Lcn2 levels. There is extensive evidence that EGFR stimulation, e.g. via binding of EGF or other stress-induced ligands such as angiostatin II binding, promotes proliferation and apoptosis resistance in renal tubular cells [11, 12]. In vitro studies by Okada et al. [13] have shown that both transforming growth factor (TGF)-β1 and EGF synergistically drive EMT in cultured tubular cells. Transgenic mice with renal overexpression of a dominant-negative EGFR show reduced renal fibrosis upon 5/6 nephrectomy, suggesting that EGFR activation actively participates in the fibrotic process [14]. Accordingly, overexpression of a constitutively active form of EGFR results in excessive proliferation of tubular epithelial cells and contributes to an increased mortality of these mice [15].

HIF-1α is already known to play a central role in EMT development and renal fibrosis [16, 17]. The study by Viau et al. is the first to define HIF-1α as an intermediate between EGFR activation and Lcn2 up-regulation, however, future in-depth analyses will be necessary to unravel the molecular mechanisms in the EGFR:HIF-1α:Lcn2 framework.

Among the increasing number of “early indicators” of AKI that have been identified over the last decade, kidney injury molecule (KIM)-1 has undergone a comparable evolution from a mere biomarker to a key player in renal injury processes. The diagnostic and predictive potential of KIM-1 that emerged from various animal models of ischemic and drug-induced kidney injury has now been confirmed by studies in humans with AKI [18–20]. In contrast to the role of Lcn2 as a downstream mediator in profibrotic pathways, KIM-1 exerts protective effects in the functional recovery mechanisms following renal damage as it transforms epithelial cells to a phagocyte-like phenotype and thereby contributes to the clearance of apoptotic cells [21].

**Take-home-message**

The carrier protein Lcn2lipocalin-2 may not only serve as a promising biomarker for AKI, but it also mirrors proliferative changes in the kidneys and progression to CKD in mice and humans. Since it acts as a downstream signalling event upon EGF receptor activation, it may also bear the potential as a therapeutic target.

**Conflict of interest statement.** None declared.


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