Introduction

Galectin-3, a member of the β-galactoside-binding lectin family, is a multifunctional protein with various biological functions. Recently, this lectin has been put forward as a possible biomarker of inflammation and renal fibrosis. Several studies have shown that the renal function plays a predominant role on the plasma concentration of galectin-3. In this review, we focus on the involvement of galectin-3 in the pathogenesis of kidney diseases as this soluble protein intervenes in the onset and development of diabetic and non-diabetic nephropathies.

General Characteristics

Structure

Galectins are carbohydrate binding proteins with similar elements and a specific affinity for β-galactosides of glycoconjugates [1–6]. Until now, 15 galectins have already been identified in mammals, which are classically divided into 3 subgroups: prototype, tandem repeat and chimeric (fig. 1a) [1, 3, 5]. Prototype galectins con-
sist of one carbohydrate recognition domain (CRD) of approximately 130 amino acids and can be found as monomers or homodimers. The monomer is characterized by a short N-terminal domain with a unique short end, continuing into an intervening proline-glycine-alanine-tyrosine-rich repeat motif and a C-terminal carbohydrate-binding domain. Tandem repeat galectins contain 2 CRDs affiliated by a peptide. Galectin-3 or Mac-2 (29–35 kDa, LGALS3, Chr 14, q21–q22) is the first and only identified chimeric galectin as it contains a collagen-like domain at the N-terminal and a CRD at the C-terminal (fig. 1b) [3]. With at least 2 possible binding sites for each CRD, galectin-3 has the capacity to oligomerize different sugars [7]. Two modes of oligomerization (type-N mode and type-C mode oligomers) have been described, depending upon the N-terminal domain of the polypeptide [8]. Although the influence of the oligomerization type on the affinity with sugar ligands still remains unclear, oligomerization of galectin-3 into higher-ordered galectin-glycoprotein complexes appears to be a central element of the biological function of the lectin [9].

**Expression**

As galectins do not have signal sequences, they are formed in the cytoplasm [3, 10, 11]. Nevertheless, their distribution is not limited to the cytoplasm, as they can also be found in the nucleus, cortex and even in the extracellular compartment. The intracellular distribution depends on the cell cycle – mainly cytoplasmic in quiescent cells and nuclear in replicating cells. A key event for galectin-3 shuttling from the nucleus to the cytoplasm is phosphorylation at the N-terminal serine 6 [12].

The presence of galectin-3 has been demonstrated during human embryogenesis and more specifically during the primary trimester of the pregnancy. Using immunohistochemistry and Western blotting, galectin-3 was prominently detected in epithelial cells from the respiratory and digestive tract, as well as in the skin, myocardial cells, specific chondrocytes and the notochord [5, 13]. During development, galectin-3 is also widely expressed in ureteric bud branches of the metanephros. In the mature kidney, it is only sparsely found in the distal tubules and in a subset of cells of the collecting ducts, the α-intercalated cells [11]. Galectin-3 exerts its effect in a
3-dimensional environment through modulation of both cell–cell and cell–substratum adhesions. The interplay between these adhesions is important for the growth of multicellular aggregates and extensions occurring during normal kidney tubulogenesis [6].

Galectin-3 has also been demonstrated in blood vessels, activated T-cells, fibroblasts, mast cells, macrophages, eosinophils and tumor cells [2–5]. Although the trophoderm cells contain galectin-3, the role of this lectin is probably not essential for implantation as galectin-3-null mutant mice are fertile and viable [14].

**Functions**

The widespread division of galectin-3 emphasizes its important role in many pathways, such as cell attachment, cell differentiation and proliferation, embryogenesis, inflammation, cancer invasion and metastasis [3, 5, 10]. However, the role of galectin-3 is not yet completely understood. The sites of galectin-3 expression change in function of the pathological processes [5]. Just like the other members of the galectin family, galectin-3 can act as a pattern recognition receptor, binding glycans on the surfaces of bacteria, viruses, fungi and protista. Galectin-3 plays a role in the inflammatory response, the immune system and tissue fibrosis [15]. When bound to glycoconjugates, the collagen-like domain empowers galectin-3 to form oligomers, by which galectin-3 can crosslink cell surface glycoproteins [2, 3, 5]. In this way, galectin-3 is involved in cell–cell and cell–matrix interactions and has been associated with cell growth, differentiation, inflammation, fibrogenesis and stiffening of the extracellular matrix (fig. 1c) [3, 6]. When not bound to glycoconjugates, galectin-3 self-associates in a manner that is dependent on the C-terminal domain and is inhibitable by the lectin’s carbohydrate ligands [16]. In the intracellular compartment, galectin-3 is involved in the transport of glycolipids and proteins destined for the apical surface of epithelial cells and regulates proliferation and apoptosis via carbohydrate-independent mechanisms [9].

Complex formation between galectin-3 and glycans or glycoproteins can be modulated by changing the pH from a neutral to more acidic environment. In comparison with Galβ1-4GlcNAc (LacNac), asialofetuin and transferrin, which lose their binding with the lectin, the binding between galectin-3 and lactose persists in acidic environments below pH 6. An efficiency spectrum of galectin-3 association with binding partners at changing pH values can serve as a tool to regulate lattice formation in cellular compartments [9]. In addition, internalized galectin-3 directs the subcellular targeting of apical glycoproteins by membrane recycling, which is a pH- and lactose-dependent process [17].

Galectins are also involved in membrane transport of transmembrane proteins. Using Madin–Darby canine kidney cells (MDCK cells), which apically expressed MUC1, a transmembrane glycoprotein involved in epithelial development and protection against pathogens, it was demonstrated that the small subunit of MUC1 bound mainly with galectin-3, while the large subunit bound to both galectin-3 and -9 [2]. Investigating the interaction between MDCK cells and extracellular matrix elements, single cell force spectroscopy showed no role for galectin-9, whereas galectin-3 influenced the adhesion of MDCK cells to collagen types I and IV [3].

**Analytical Methods**

An enzyme-linked immunosorbent assay (ELISA) has been developed for the determination of galectin-3. In this 2-site immunoassay, the capture antibody, a rat IgG2a monoclonal antibody raised against mouse galectin-3 protein, is immobilized on 96-well plates. The N-terminal portion of the human galectin-3, where the epitope for the assay is located, is 100% homologous with the murine galectin-3. The within-run precision is <8%. Total coefficient of variation (CV) at the low galectin-3 concentration of about 6 ng/ml is <10%, total CV at the midlevel concentration of 21 ng/ml is approximately 7%, and total CV at the high level of approximately 70 ng/ml is about 15%. The galectin-3 assay is linear from the limit of quantitation at 0.96–130 ng/ml. The performance characteristics of this assay are appropriate for clinical measurement using either serum or EDTA plasma specimens. Stability and specificity data showed that the ELISA is robust from cross reactivity and interference studies [18].

Recently, a European multicenter evaluation of the automated galectin-3 assay on the Abbott ARCHITECT immunoassay instruments (a chemiluminescent microparticle immunoassay) has been performed. The total assay CV was 2.3–6.2% and 1.7–7.4% for the routine and STAT assays, respectively. Both assays demonstrated linearity up to 120 ng/ml. Higher galectin-3 concentrations were measured in plasma samples than in serum samples. A good correlation was found with the galectin-3 ELISA. The reference interval for healthy individuals was 25.2 (upper 95th percentile) and 28.4 ng/ml (97.5th percentile), respectively. Significantly lower values were found in subjects <50 years [19].

The VIDAS® automated enzyme-linked fluorescent assay is another method to measure the galectin-3 concentrations. An excellent agreement with the refer-
ence ELISA assay has been demonstrated ($r = 0.90, p < 0.001$). The between-run CVs obtained with pools of serum were 4.5 and 3.9% at 15 and 31 ng/ml, respectively [20].

**Galectin-3 and Renal Function**

**Acute Renal Failure**

Acute renal failure (ARF) is the abrupt and sustained decrease in renal function resulting in retention of nitrogenous and non-nitrogenous waste products [21]. Several definitions and classification systems of ARF have been proposed, based on changes in serum creatinine concentration or changes in estimated glomerular filtration rate (eGFR) from a baseline value and changes in urine output per kilogram of body weight over a specified time period [22]. Although treatments have been evolving, mortality rates are still high. Recently, apoptosis and inflammatory processes are put forward as possible causes of ARF. Inducing an ischemia/reperfusion renal injury (IRI) in rats with and without nephrotoxic folic acid showed that galectin-3 mRNA expression increased at 2 h and elevated up to 48 h. A highly significant negative correlation was found between the galectin-3 mRNA expression and the serum creatinine levels. In addition, galectin-3 expression was localized in the proximal tubules and extended to the more distal side after reperfusion. Based on these findings, an important role for galectin-3 in acute tubular injury and the following regeneration stage has been suggested [23].

**Chronic Renal Failure**

Besides its role in acute kidney injury, galectin-3 is also involved in the pathogenesis of chronic kidney disease (CKD). In 133 subjects with chronic heart failure, a strong correlation was found between the plasma galectin-3 concentration and indexes of renal dysfunction, including cystatin C levels [24]. This finding was confirmed in the Framingham Heart Study with 2,450 participants (mean age 57 years, 53% females, mean follow-up of 10.1 years). Higher serum galectin-3 concentrations were associated with an increased risk of incident CKD, even after adjustment for known clinical predictors of CKD, as well as for circulating biomarkers known to enhance CKD prediction. A similar strong correlation was observed with galectin-3 over 10 years of follow-up. No association was observed between the albuminuria level and the serum galectin-3 level [25]. This was in contrast to the independent association between the serum galec-

- tin-3 concentrations and microalbuminuria in patients with chronic heart failure in the Cardiovascular Health Study [26].

As the clearance of galectin-3 appears to be primary hepatic, galectin-3 may be helpful for the identification of subjects at risk for the development of CKD many years before clinical onset. In addition, this lectin plays an important role in the fibrosis process, which takes place early in the pathogenesis of CKD [25]. However, in a community-based multicenter cohort of 2,763 older adults free of clinical heart failure (mean age 72 ± 5 years, 63% women), higher serum galectin-3 concentrations were not associated with a 30% decline in eGFR or the development of incident eGFRs <60 ml/min/1.73 m² after adjustment for potential confounders and other cardiac biomarkers. The fact that this study comprised older adults with a higher burden of morbidity with relatively preserved kidney function may explain the different results with other studies [27].

Several small studies have investigated renal handling of galectin-3 in patients with end-stage renal disease. In a small cross-sectional study of 101 heart failure patients, 105 anuric hemodialysis patients and 20 healthy subjects, strongly elevated plasma galectin-3 concentrations were detected in patients with terminal renal insufficiency in comparison with controls [28]. A similar result was found in a study with 100 hemodialysis patients, 50 predialysis patients and 94 healthy individuals [29]. In a third study of 88 hemodialysis patients with a follow-up period of 22.2 ± 4.7 months, a multivariable Cox proportional hazards model showed that a plasma galectin-3 cutoff point of 23.73 ng/ml was an independent predictor of all-cause mortality [30].

**The Role of Galectin-3 in Different Renal Pathologies**

**Diabetic Nephropathy**

Advanced glycation end products (AGEs) are formed by non-enzymatic glycation and play an important role in the development of diabetic nephropathy [31]. Being an AGE binding protein and a receptor for advanced lipid oxidation end-products (ALE), galectin-3 has been put forward as an important mediator in diabetic nephropathy. Galectin-3 is involved in the assembly of AGE-specific cellular receptor complex components. It assists in the efficient cell surface attachment and endocytosis by macrophages of a heterogeneous pool of AGE-modified senescent macromolecules with diverse affinities. In this way, it contributes to the elimination of these pathogenic...
substances [32, 33]. This finding has been supported by the fact that galectin-3 knockout (KO) mice developed accelerated diabetic glomerulopathy in comparison with wild-type (WT) animals [32, 34, 35]. Deficiency of galectin-3 leads to an increased impairment of the vessels and the kidney, due to a decreased removal of AGE and ALE [32, 34]. Using immunohistochemistry, the number of galectin-3-positive cells in glomeruli closely correlated with the progression and prognosis of diabetic nephropathy [36].

In 2 large cohorts of patients, the German Diabetes Mellitus Dialysis (4D) study (1,168 dialysis patients with type 2 diabetes mellitus) and the Ludwigshafen Risk and Cardiovascular Health (LURIC) study (2,579 patients with coronary angiograms), the association among renal function, adverse outcomes and galectin-3 was investigated over the entire spectrum of kidney function, ranging from normal in the LURIC study to patients with ESRD in the 4D study. The patients were divided into 3 groups, depending on their renal function: eGFR <60, 60–89 and >89 ml/min/1.73 m². The circulating serum/plasma galectin-3 concentrations increased with a declining kidney function (table 1). Several hypotheses have been postulated to explain this finding, for example, an at least partly clearance of galectin-3 by the kidneys. In addition, serum galectin-3 levels were significantly associated with clinical end points of all-cause mortality, cardiovascular mortality, death due to infection and sudden cardiac death in patients with an eGFR of 60–89 ml/min/1.73 m² [37]. The link with a worse outcome due to infections can be explained by the important role of galectin-3 in infection and inflammatory response with neutrophils, T cells, monocytes and mast cells [38]. In patients with an even lower eGFR of <60 ml/min/1.73 m², this novel biomarker was associated with myocardial infarction and death due to chronic heart failure. Although galectin-3 expression has been demonstrated in atherosclerotic plaques, its role in atherosclerosis may be ambiguous. In the group of dialysis patients, similar findings were observed, although there was not a significant association between galectin-3 and the incidence rates of myocardial infarction and sudden cardiac death. Although this study suggests the use of galectin-3 for risk stratification of patients with renal insufficiency, the lack of a ‘gold standard’ for risk stratification in renal disease weakens this statement [37].

The renin-angiotensin system and the receptor for AGEs (RAGE) potentiate diabetes-associated atherosclerosis. Treating diabetic male RAGE/apolipoprotein E double KO mice with quinapril abolished the development of experimental diabetes-associated atherosclerosis, significantly attenuated diabetes-associated increases in fibrosis and inflammation and significantly attenuated the diabetes-induced increase in galectin-3 [39]. Albuminuria is characterized by a great loss of leukocytes, especially B cells, but also subpopulations of macrophages and CD4+ T cells. The surviving macrophages are an M2-like subpopulation with a decreased expression of the M1 marker and increased expression of galectin-3. Administration of enalapril to the db/db mice and the streptozotocin-induced diabetic nephropathy models with established disease resulted in a decreased albuminuria and a repopulation of M1-like phenotype macrophages and an expansion of CD4+ and CD8+ T cells [40].

<table>
<thead>
<tr>
<th>Table 1. Distribution of serum/plasma galectin-3 concentration in function of CKD stages</th>
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<tr>
<td>CKD stage, ml/min/1.73 m², mean ± SD</td>
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<tr>
<td>-------------------------</td>
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<tr>
<td>eGFR, ml/min/1.73 m²</td>
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<tr>
<td>≥90</td>
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<tr>
<td>60–89</td>
</tr>
<tr>
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<td>30–44</td>
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<td>Hemodialysis</td>
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Galectin-3 and Kidney Diseases
Renal Fibrosis

Renal fibrosis is characterized by activation of fibroblasts and myofibroblasts, endoplasmic reticulum stress, microvascular rarefaction and tissue hypoxia and is an almost inevitable consequence in CKD. The onset of renal fibrosis is associated with diabetes mellitus and atherosclerosis in developed countries. Macrophages are the key cells in renal fibrosis. Galectin-3 is secreted by activated myofibroblasts in renal, liver and cardiac fibrosis and has recently been put forward as a marker of fibrosis [41]. Obstructive nephropathy is also involved in the onset of renal fibrosis, which is facilitated by leukocytes. This leukocyte recruitment is mediated by RAGE in cooperation with intercellular adhesion molecule 1. This process is possibly mediated via macrophage-derived galectin-3 secretion, which is a major profibrotic stimulus within the kidney [42]. In mice with CKD induced by UUO, the galectin-3 expression in kidney tissue was significantly higher than in WT mice. A profibrotic signaling axis between macrophages and tissue fibroblasts has been suggested as secretion of galectin-3 by macrophages resulting in accumulation of myofibroblasts, in conversion of renal myofibroblasts to a profibrotic phenotype and in collagen deposition. As renal fibrosis progresses, an upregulated galectin-3 expression and a continued increase of macrophage recruitment are observed. Transforming growth factor-beta (TGF-β)-dependent and -independent mechanisms are involved in the pathogenesis of renal fibrosis. Despite comparable renal expression of TGF-β and intact TGF-β signaling to Smad 2 and Smad 3 in WT and galectin-3–/– mice, absence of galectin-3 markedly inhibited TGF-β signaling to Smad 2 and Smad 3 in WT and galec-3-null mice showed less interstitial fibrosis after transplantation than galectin-3-positive mice, which indicates a role of galectin-3 in the onset of interstitial fibrosis [43].

In contrast to the previous findings, Okamura et al. [45] suggested a protective role of galectin-3 in renal fibrosis. In mice with an UUO, galectin-3-deficient mice showed a more severe degree of renal damage in comparison with WT mice. Galectin-3 did not influence the biomarkers of inflammation, but decreased apoptosis in renal tubular cells and modulated the extracellular matrix, which led to restrained fibrosis and improved remodeling. Intracellular galectin-3 protects renal tubules independently of the carbohydrate domain. Extracellular galectin-3 may direct Endo180-mediated collagen degradation through its carbohydrate domain. Taken together, the role of galectins and especially galectin-3 in renal fibrosis is still controversial [45, 46].

Renal Ischemia

IRI is an inflammatory syndrome where innate and adaptive immune responses participate. Galectin-3 plays an important role in both early and late stages of inflammation. Investigating the role of galectin-3 in the inflammation triggered by IRI in a mouse model, Fernandes Bertocchi et al. [4] showed that the absence of galectin-3 is associated with a reduced extent of renal injury through a diminished macrophage chemotaxis. A lower expression of mRNA of monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-1β and reactive oxygen species (ROS) production was observed. MCP-1, a chemokine related to the function of galectin-3, is implicated in chemotactic phagocytes into the tissue. Macrophages are involved in tissue aggression and repair following IRI, secreting IL-6 and IL-1β, some of their important proinflammatory products. Finally, ROS are produced by neutrophils and macrophages during ischemia and released after reperfusion in part because of the cleavage of arachidonic acid by cyclooxygenases. ROS promote overexpression of adhesion molecules, pro-inflammatory genes, chemokines and cytokines from macrophages. So in the absence of galectin-3, a decreased macrophage function in association with decreased concentrations of pro-inflammatory cytokines, chemokines and ROS will result in a reduced inflammation.

Galectin-3 may play an important role in adapting cortical collecting ducts to metabolic acidosis by the adaptation of β-intercalated cells, which regulate the acid-base transport. During metabolic acidosis, these cells show a significantly higher concentration of galectin-3 [10]. The adaptation process is characterized by polymerization and deposition of the extracellular matrix protein hensin, a process which is enhanced by galectin-3 [1, 10]. The interaction between the CRD-domain of galectin-3 and hensin is critical for polymerization of hensin [1]. Galectin-3 may also be involved in the complex process of kidney regeneration following IRI [47].

Cardiorenal Syndrome

The increased prevalence of diabetes mellitus, hyperlipidemia and hypertension in developed countries has resulted in a higher percentage of patients with CKD and cardiovascular disease. A positive correlation has been demonstrated between advanced loss of renal function and the cardiovascular complication rate [48]. The cardiorenal syndrome type 4, also referred to as CKD cardiomyopathy, is a process in which CKD stimulates heart failure by changing the myocardium [49]. Left ventricular hypertrophy, cardiac fibrosis, coronary atherosclerotic...
heart disease, accelerated coronary calcification and also a greater risk of sudden death have a proven correlation with CKD. The reversed association between cardiovascular morbidity and limited GFR is evident, but there is little known about the underlying process of this association. Fibroblast growth factor 23, uremia, cardiac myocyte–capillary mismatch and galectin-3 have been proposed as important mediators in the rise of this cardiovascular risk [48].

Galectin-3, released by macrophages, binds to cardiac fibroblasts with ventricular dysfunction and an increased collagen production as a consequence [50]. Galectin-3 and TGF-β are important in the communication between interstitial cells, which results in upregulation and proliferation of fibroblasts and myofibroblasts [49]. Elevated serum galectin-3 levels are correlated with serum markers of cardiac extracellular matrix turnover in patients with heart failure [51]. Investigating the relationship of plasma galectin-3 to the renal function in patients with heart failure showed an inverse relationship between galectin-3 and GFR, independently of the presence of heart failure [52].

Polycystic Kidney Disease

During nephrogenesis, galectin-3 has been demonstrated in derivatives of the collecting ducts and in the branching ureteric buds [53]. Galectin-3 is a key regulator in the terminal differentiation and in the growth of collecting ducts [54, 55]. Therefore, galectin-3 may be involved in the pathogenesis of autosomal recessive polycystic kidney disease (ARPKD), which is associated with hepatorenal congenital fibrocystic syndromes [56]. Galectin-3 possibly blocks the expansion of kidney cysts, confirmed by a higher expansion of cysts in the presence of galectin-3 antibodies [6, 57]. Investigating pre- and postnatal babies with ARPKD showed that the cystic epithelium was generally positive for an immature form of galectin-3, which was located in the cytoplasm in majority of the cases [11]. The correlations found between galectin-3 expression and the development of kidney cysts underline the role of galectin-3 in epithelial growth and differentiation [57].

Assuming that galectin-3 influences the growth of cysts in ARPKD, galectin-3 expression in kidneys of mice with congenital polycystic kidney (cpk) has been investigated. After an excessive exposure of WT galectin-3 in cpk cysts in a suspension culture, a statistically significant diminution in the number of cysts (60–80%) was observed after 24 h. In addition, the development of cysts in galectin-3-null mutants and WT in vivo was compared. Both developed common medullary cysts, but the cystic index in the cortex was significantly raised in the galectin-3-null mutants compared to the WT mice. After administration of 150 μg paclitaxel, a decrease of galectin-3 levels was observed, which resulted in a longer life span of the WT cystic mice. The reduced percentage of cystic epithe-lia overall and/or potential redistribution of galectin-3 might explain this phenomenon [58].

Renal Cell Carcinoma

Renal cell carcinoma (RCC) accounts for 3–4% of the malignancies in adults with an increasing incidence. RCC has a higher incidence in males and tops out at the 6th and 7th decade of life. In one-third of all patients, RCC is already metastatic or locally advanced at time of diagnosis [59]. Significantly higher galectin-1 and -3 levels in tumor tissue have been detected in comparison with the nontumorous surrounding tissue by mRNA expression analysis [60–62]. Logistic regression analysis showed that male gender is significantly correlated with higher levels of galectin-1 and -3 in clear-cell RCC. These gender differences in galectin concentration were only found in the RCC tissue and not in the surrounding tissue [63]. Galectin-1 and -3 have been put forward as potential tumor markers for RCC with a sensitivity and specificity of 47 and 98%, respectively [60].

A strong overexpression of galectin-3 has been reported in chromophobe RCC and oncocytoma, with positive immunohistochemical detection of galectin-3 in 90 and 96% of the samples, respectively. This emphasizes the potential role of galectin-3 in the differential diagnosis of RCC [64]. This has been supported by a tissue microarray immunohistochemical study, showing that carbonic anhydrase IX, galectin-3, CK7 and α-methylacyl coenzyme-A racemase were sensitive and specific for the differential diagnosis of renal epithelial tumors. In comparison with chromophobe RCC and oncocytoma, a significantly lower staining extent and intensity of galectin-3 was found in clear-cell RCCs and in papillary RCCs [65]. The presence of galectin-3 has also a prognostic value as galectin-3 detection was positive in 55.1% of Fuhrman nuclear grades 3 and 4 and only in 22.7% of Fuhrman nuclear grades 1 and 2 [64]. However, the study by Sakaki et al. [62] did not find any significant difference in galectin-3 expression between grades 1 and 2 and between grades 2 and 3. The chance of progression of metastasis of a clear-cell RCC is lower in patients with low galectin-3 levels, as this lectin inhibits cell–extracellular matrix and cell–cell interactions [61]. Significantly higher plasma galectin-3 levels have been measured in RCC patients with remote...
metrical collecting ducts segments was significantly greater overall number of galectin-3 positive medullary and cortical tubules. In the non-responding steroid group, the collecting ducts of the cortex and to a lesser degree in distal tubules demonstrated in renal medulla and cortex, especially in parts of the mesangial matrix. In a later phase, followed by collagen IV and laminin deposition. It also showed synergic stimulating effects of TGFB on matrix development.

Kidney Transplantation
As galectin-3 plays an important role in the start of kidney fibrosis, the involvement of this lectin has been investigated in the development of chronic allograft injury (CAI). CAI is characterized by tubular atrophy and interstitial fibrosis. In a murine model of CAI, a significant tubular atrophy, interstitial fibrosis and to a lesser extent an increase of galectin-3 was observed in C57BL6, galectin-3-positive mice after transplantation in comparison with the control syngeneic group. In galectin-3-null mice, a significant lesser interstitial fibrosis and a conservation of tubules was observed. In addition, no difference in leukocytes infiltration could be demonstrated in contrast to a diminished expression of YM1, a marker of alternative macrophage activation, besides a reduced number of CD4+ T cells.

Investigation of the serum levels of galectin-3 in renal transplant patients and in a control group showed significantly elevated concentrations of galectin-3 before transplantation. Three months after the kidney transplantation, the serum galectin-3 levels were significantly reduced.

Familial Mediterranean Fever
Familial Mediterranean fever (FMF), an autosomal recessive and auto-inflammatory disease, is frequently associated with AA amyloidosis. Through the use of colchicine, a decrease in the occurrence of FMF-associated AA amyloidosis has been observed. The early identification of amyloidosis, based on proteinuria, is very important. As significantly higher serum galectin-3 concentrations have been measured in patients with FMF, this lectin may play a role in the pathogenesis of
Galectin-3 and Kidney Diseases

Infectious Diseases

In chronic infectious disease such as hepatitis C and HIV, elevated serum concentrations of galectin-3 have been reported [77–79]. Viral load and expression of galectin-3 are positively correlated in HIV-1 infected patients [79]. An acute hantavirus infection is also associated with an upregulation of galectin-3. Using African green monkey kidney epithelial Vero E6 cells infected with the apathogenic hantavirus (Prospect Hill virus (PHV) and Tula virus (TULV)) and cells infected with the pathogenic Puumala virus (PUUV), the induction of galectin-3 was compared with a group of normal Vero E6 cells. Real-time PCR showed no elevation of galectin-3 in the control cells. On the other hand, the cells infected with PHV, PUUV and TULV expressed galectin-3. Immunoblotting demonstrated an increase in galectin-3 on the protein level. The hantavirus-induced increase of galectin-3 seems to interfere with the expression of the viral N protein, which is an important step in the gene expression of viruses, indicating that the antiviral response requires galectin-3. The mechanism of this antiviral activity is not fully understood, but 2 theories are provided. The first possibility withholds the capacity of galectin-3 to attach directly to the Hantan viruses. The other possibility is an activation of the innate immune system by activating the complement system. In line with this theory, a parallel increase of galectin-3 and activation of the complement system could be demonstrated in patients infected with the PUUV. In another part of the study, the upgrade of galectin-3 in human umbilical vein endothelial cells was knocked down by using interfering RNAi. This resulted in an increase of the viral nucleocapsid protein, suggesting that galectin-3 negatively affects the virus replication [80].

The role of galectin-3 has also been investigated in bacterial infections, by examining the occurrence of galectin-3 in case of a Proteus mirabilis infection, which often causes urinary tract infections. The bacterium adheres with non-agglutinating fimbriae to different cell lines, including MDCK cells. On one hand, monoclonal anti-Mac-2-antibodies were used to prove its manifestation on the extracellular surface of the plasma membrane. On the other hand, pre-incubation with those specific monoclonal anti-Mac-2 antibodies inhibited Proteus mirabilis to bind to MDCK cells, which suggests the key role of galectin-3 for the adhesion of the bacterium. Incubation with anti-NAF-antibodies, used as a control, showed no positive staining [81].

Experience with Galectin Inhibitors

Galectin-3 emerges as a potential therapeutic target in the treatment of interstitial fibrosis and glomerulopathy (table 2). Galectin-3 inhibitors, such as N-acetyllactosamine, have shown potential to improve hypertensive nephropathy in rats. Hypertensive nephroangiosclerosis is characterized by hyperfiltration, ischemia, apoptosis and genetic predisposition [82]. In transgenic REN2-con rats with high blood pressure, a hypertensive nephropathy was observed based on an injury of glomerular endothelial cells, exaggerated production of the extracellular matrix and microinflammation. Microinflammation was demonstrated by a significantly elevated amount of interstitial macrophages in the transgenic rats. After administration of the galectin-3 inhibitor, a decrease in proteinuria, reduced renal damage and enhanced renal function were observed. In the control group of Sprague–Dawley rats, none of the considered parameters for hypertensive nephropathy changed after administering N-acetyllactosamine [83]. In a murine model of steatohepatitis, non-alcoholic steatohepatitis mice were treated with GR-MD-02, a drug that inhibits galectin-3. In the kidney tissue, a lower percentage of fibrosis and a less frequent and less severe mesangial expansion were observed [84].

The blocking effects of modified citrus pectin (MCP) on galectin-3-performance have been suggested. In vitro studies have demonstrated the galectin-3 blocking effects with MCP on cell adhesion and chemotaxis [85, 86]. MCP is a derivative of pectin which can bind to the galectin-3 CRD and thereby predominantly antagonists function linked to this role [87, 88]. The effects of MCP as an in-
hibitor of galectin-3 have been investigated on acute kidney injuries. Therefore, male mice were divided into 3 groups. The first group was instilled with sodium bicarbonate intraperitoneal and supplied with regular drinking water. Folic acid nephropathy was induced in the second and third group, with the second group receiving regular drinking water and the third group receiving 7 days 1% modulated citrus pectin in the water. At the start, all mice with induced nephropathy had enlarged kidneys and suffered from weight loss. These developments were significantly inferior in the MCP treated group, but MCP did not induce a significant decrease in galectin-3. MCP lowered the cell proliferation, but did not influence apoptosis. At a later stage, renal fibrosis, apoptosis, cytokine level, macrophages and galectin-3 level were reduced in mice treated with MCP [89].

DX-52-1 and HUK-921 bind galectin-3 outside of its beta-galactoside-binding site. In comparison with DX-52-1, which is a more potent inhibitor of cell migration than DX-52-1, HUK-921 had a far greater selectivity for galectin-3 over radixin, both in vitro and in cells. Treatment of galectin-3-overexpressing MDCK cells with DX-52-1 or HUK-921 resulted in a change in localization of GFP-galectin-3 and reversion of the galectin-3-overexpressing cells from a highly spread state to a more normal epithelial morphology. The data suggest that DX-52-1 and HUK-921 inhibit a carbohydrate binding-independent function of galectin-3 that is involved in cell migration [90].

By binding on a mineralocorticoid receptor, aldosterone regulates the electrolytic balance and blood pressure. As demonstrated in rat experiments with hyperaldosteronism, galectin-3 rose under the influence of an aldosterone increase, which was associated with cardiac and renal fibrosis and dysfunction. As inhibitors of aldosterone (spironolactone) and inhibitors of galectin-3 (modified citrus pectin) returned the parameters back to a normal level, galectin-3 could be a new biotarget for specific pharmacological interventions [91].

**General Conclusion and Clinical Perspectives**

Because of the growing importance of CKD as a global public health problem, there is a call for the identification of regulating key players in renal inflammation and subsequent fibrosis. As illustrated in this review, galectin-3 is involved in the pathogenesis of several kidney diseases by promoting macrophage migration, myofibroblast activation and collagen synthesis. However, the underlying signaling pathways should be further unraveled.

Galectin-3 can probably not be used as a diagnostic (screening) biomarker of CKD due to a lack of sensitivity and specificity, but may have the potential to predict a progressive decline in kidney function. Therefore, novel large prospective clinical trials should investigate and validate the relationship of this multifunctional lectin with

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**Table 2. Investigation of galectin-3 inhibition in animal models**

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<tr>
<th>Kidney disease</th>
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References


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