CD44, TLR4, TREM-1/DAP12 in renal injury, inflammation and fibrosis

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Summary and Discussion

CD44 in shock-associated renal damage and inflammation

Septic shock remains a major cause of death in intensive care units, with an estimated mortality of approximately 50%.[1, 2] Acute kidney injury (AKI) is one of the most important forms of organ dysfunction encountered in sepsis as it increases the severity of the illness, mortality, complexity and duration of patient care.[3]

In the study described in **chapter 2**, we used LPS to induce a systemic inflammatory response and the associated renal pathological alterations. LPS has been widely used in animal models to study the pathomechanisms underlying systemic immune reactions and related organ failures, and it mimics many of the initial clinical features of human sepsis.[4-7] Nevertheless, LPS administration is not an exact model of sepsis as bacterial outgrowth does not occur and only Gram$^-$ bacteria possess LPS. However, it remains a good and well reproducible model of SIRS.

Mostly using animal models, a crucial role of CD44 has been demonstrated in a variety of inflammatory diseases,[8-12] including endotoxemia.[13-16] In chapter 2, we demonstrated that upon LPS exposure, lack of CD44 impairs the early proinflammatory cytokine response, inflammatory cell migration/chemotaxis, endothelial activation, and consequentially delays the onset of renal dysfunction. After LPS injection, CD44 expression, which is generally absent in normal adult kidneys, was induced in wild-type kidneys together with its ligand HA. CD44$^+$ cells were detected mainly in the interstitium and some in the glomeruli, but not among tubular cells, in contrast to what is seen in other renal pathological conditions.[17-20]

The lower proinflammatory cytokine levels in plasma and particularly in the kidneys of CD44-null mice after LPS challenge suggest that CD44 might amplify the inflammatory responses initiated by mononuclear cells. Stimulation of bone marrow-derived macrophages with LPS showed a diminished activation of the NF-κB pathway in CD44 KO macrophages and a lower expression of proinflammatory cytokines induced by the MyD88- or Trif-pathways. Furthermore, CD44 cross-ligation on wild-type macrophages significantly increased TNF-α secretion upon LPS. The CD44-mediated rise in cytokine production was inhibited by blocking the p38 MAPK or PI3K/Akt pathways prior to LPS exposure. Engagement of CD44 can activate the PI3K/Akt, MAPK/ERK, p38 MAPK pathways.[21-23] These pathways are also involved in LPS-elicited activation of NF-κB (PI3K/Akt, MAPK) and AP-1 (MAPK), and LPS-uptake via the CD14/TLR4/MD-2 complex (p38 MAPK).[24-30] Supposedly, *in vivo* CD44 interacts with HA (LMW), osteopontin, or cytokines such as MIP-1β contributing to the magnitude of inflammation. CD44 may also contribute to SIRS by interacting with MIF (macrophage migration inhibitory factor) and its receptor CD74 in a complex that leads to signal transduction.[31, 32] MIF is an important mediator of sepsis;[33] MIF promotes proinflammatory responses by amplifying cytokine secretion through the upregulation of TLR4 expression and, at the same time, inhibits the anti-inflammatory effects of endogenous glucocorticoids of the endocrine system.[34-38]

Of particular interest is the elevated production of the anti-inflammatory IL-10 observed in absence of CD44 both *in vivo* and *in vitro*. A plausible regulator of this phenomenon is heme oxygenase-1 (HO-1), which is induced by LPS and provides defense against endotoxemia, includ-

ing LPS-induced ARF, by controlling the IL-6/IL-10 balance.[39, 40] From our observations, we know that renal expression of HO-1 was enhanced after LPS-injection, and at 24 hours HO-1 mRNAs and proteins were significantly higher in CD44 KO kidneys than in wild-type kidneys. It remains, however, difficult to explain the link between lack of CD44 and high HO-1 expression. In a murine model of acute liver injury induced by intravenous injection of Propionibacterium acnes and LPS, Gu and co-authors found that administration of an HO-1 inducer alleviates hepatic failure through suppression of adaptive immune responses. Indeed, HO-1 inhibited the activation and proliferation of hepatic $CD4^+$ T cells and reduced the level of CD44 expression on these cells.[41] Yet, no studies describe the influence of CD44 on HO-1 expression.

CD44 is also known for its functions in promoting migration, adhesion and rolling of leukocytes.[42] This notion together with the reduced MCP-1 production and the lessened VCAM-1 induction in CD44 KO kidneys can explain the attenuated renal recruitment of leukocytes in absence of CD44. Inducible NO synthase (iNOS) expression is particularly relevant in this setting, as various animal studies have demonstrated that selective iNOS inhibition attenuates sepsis-induced renal dysfunction and improves survival.[43-47] In wild-type mice, renal iNOS levels were significantly increased as soon as 2 hours after LPS injection, whereas in the CD44-deficient mice this upregulation occurred only at 24 hours. In the kidneys, iNOS is a mediator of capillary dysfunction and renal injury upon LPS.[6, 48, 49] Wu *et al.* showed that following LPS administration, the induction of renal iNOS was parallel to the time course of peritubular capillary dysfunction, which is an early event that induces tubular stress and precedes renal failure.[48] In the kidney, NO acts as a chronic antagonist of various vasoconstrictors rather than as a primary vasodilator.[50, 51] Constitutive NOS isoforms are susceptible to inhibition by elevated levels of NO and some studies have suggested that sepsis might cause an iNOS-dependent decrease in endothelial NOS activity, which results in increased renal vascular resistance and, hence, reduced renal blood flow/GFR during sepsis.[6, 49]

Although many reports describe CD44 involvement in inflammatory diseases, there are studies that highlight an anti-inflammatory role of CD44 since it can contribute to the termination of inflammation by clearing apoptotic neutrophils and hyaluronan fragments from the site of injury.[52, 53] In some studies, CD44 has been described as a negative regulator of inflammation and TLR signaling, since its expression promoted the induction of TLR4 negative regulators following Klebsiella pneumoniae infection, and intratracheal and intraperitoneal LPS treatment.[54-56]

In addition, Muto and co-authors showed that HA injection into mice prior LPS administration induce "endotoxin tolerance" through the engagement of CD44 by HA.[56] Hyaluronan can be recognized by a receptor complex composed of CD44, TLR4, and MD2.[57] The consequential NF-κB activation and secretion of proinflammatory cytokines lead to desensitization of immune cells to the subsequent challenge with LPS through upregulation of A20, a negative regulator of TLR4 signaling.[56] In our study, the proinflammatory cytokine levels in plasma, kidneys, or macrophage supernatants became similar between the genotype-groups at 24 hours. Hypothetically, this might be caused by the "immunoparalysis" triggered by excessive stimulation (by LPS, HA, and/or other ligands) in wild-type cells, which in turn stop responding to stimuli and behave like CD44 KO cells.

Innate receptors in progressive renal injury

Regardless of the primary cause of the pathology, chronic renal diseases ultimately result in progressive interstitial fibrosis, which is often caused by non-resolving inflammation after a sustained injury.[58]

The toll-like receptor family serves an important regulatory role in innate immunity. TLRs act as safeguards of tissue structural integrity being activated by molecular indicators of infection (bacterial, fungal, viral) and tissue damage (DAMPs). The literature shows that TLRs promote wound healing and fibrosis in many organs affected by chronic inflammation, including kidneys, liver, heart, lungs and skin.[59] This is not surprising as inflammation and wound healing are tightly linked process; e.g. both macrophages and fibroblasts are recruited and necessary for tissue repair and debris removal.[59]

In **chapter 3**, we provide evidence for the role of TLR4 expression in the kidneys, particularly on tubular cells and myofibroblasts, in the pathogenesis of renal fibrosis induced by unilateral ureteral obstruction. TLR4 is widely and constitutively expressed in the kidneys, not only on resident dendritic cells and passenger leukocytes, but also on tubular epithelial cells.[60] After UUO, renal expression of TLR4 and of its endogenous ligands HMGB-1, GP96 and biglycan significantly increased, suggesting the occurrence of DAMPs/TLR4 interactions.

Previous studies demonstrated the detrimental effects mediated by TLR4 on renal histology and function during ischemia/reperfusion- and cisplatin-induced AKI, through the initiation of an exaggerated inflammatory response in the injured kidneys.[61-63] Following ureteric ligation, absence of TLR4 unexpectedly resulted in more severe tubular damage with more apoptotic tubular cells at day 1, similar levels of KC and MCP-1 and no differences (except for day 3) in macrophage influx as compared to the renal setting of wild-type mice. The sharp contrast with the above mentioned models of AKI implicates that TLR4 activation differentially modulate the outcome of renal injury during acute and chronic renal injury. These contrasting data might also be explained by temporal differences in the leukocyte recruitment between the two kidney injury models: ischemia/reperfusion injury leads to a massive and rapid recruitment of macrophages and moreover of neutrophils (day 1), whereas upon UUO the accumulation of the leukocyte subsets become apparent at later stage (day 3, 5).[61, 62, 64] Similarly, the pattern of TLR4 expression differs in the two models; one day of reperfusion is sufficient to induce a prominent upregulation of TLR4 in kidneys (TEC included),[62] while in the UUO study TLR4 was significantly enhanced from day 3 of obstruction. Although the wild-type TLR4 expression was not upregulated in respect to control, intact TLR4 clearly protected tubular cells from severe damage at day 1 of UUO. TLR4 can induce cell survival and proliferation and expression of adhesion molecules;[65-67] hence, a physiological basal TLR4 expression seems to protect tissue integrity from initial injury.

TLR4-deficiency markedly prevented collagen deposition at day 7 and 14 after UUO, without affecting myofibroblast accumulation. By evaluating the renal levels of active/latent matrix metalloproteinases 2 and 9, TGF-β1 and HGF, we rolled out the possibilities of an augmented matrix degradation by MMPs or of a dysbalance in pro-/anti-fibrotic growth factors in TLR4 KO mice.

We did, however, find that TLR4 KO cells are less susceptible to TGF-β1. Indeed, the TGF-β1-induced upregulation of type I collagen gene expression was completely absent in primary TEC and significantly attenuated in primary myofibroblasts derived from TLR4-null kidneys. Furthermore, the mRNA levels of Bambi in ligated kidneys were significantly higher in absence of TLR4. Bambi (BMP and activin membrane-bound inhibitor) is a transmembrane glycoprotein that lacks the intracellular kinase domain, and it is structurally related to the TGF-β type I receptors. Bambi functions as a decoy type I receptor that antagonizes TGF-β-family signals by preventing the formation of active receptor complexes upon ligand binding.[68] A link between Bambi and TLR4 has been proven by the Nature study of Seki *et al.*, which demonstrated that TLR4 ligands promote hepatic fibrosis and suppress Bambi expression through MyD88 and NF-κB. With elegant experiments, the authors showed that TLR4 activation sensitized hepatic stellate cells to TGF-β signaling through downregulation of Bambi.[69] Bambi-elicited restrain of TGF-β-mediated profibrogenic signals was also shown in a recent study on myocardial fibrosis.[70]

The later work of Campbell and co-authors support our findings on TLR4 as mediator of renal fibrosis. In fact, renal total collagen was not augmented after one week of UUO in TLR4 hyporesponsive mice (Lps-d). However, in contrast to our study, Campbell *et al.* also reported more FSP-1+ fibroblasts and less E-cadherin expression in the obstructed kidneys (1 week) of mice with intact TLR4 signaling. Unfortunately, no data are given on TEC proliferation/apoptosis or damage that could confirm or not the TLR4-mediated protection of TEC observed in our model.[71]

In conclusion, our and other studies highlight the fact that TLR4 functions go beyond innate immunity; TLR4 acts as a sensor of tissue homeostasis disruption and as a modulator of fibrogenesis when the damaging stimuli persist.

In **chapter 4**, we investigated the potential contribution of TREM-1 and DAP12 in the inflammatory and fibrotic processes induced by chronic renal damage. TREMs are cell surface immunoglobulin domain receptors that lack cytoplasm signaling motif but associate with the signaling adaptor DAP12.[72] TREM-1 was initially characterized in infections as an amplifier of inflammation and a co-activator of TLR pathways.[72-75] Recent studies support its implication also in noninfectious acute and chronic inflammatory disorders such as acute pancreatitis, ischemia/reperfusion-induced gastrointestinal injury, arthritis, inflammatory bowel disease and colitis.[76-81] We, therefore, hypothesized that activation of the TREM-1/DAP12 pathway would positively modulate the magnitude of renal inflammation and, possibly, fibrosis through the cross-talk with the TLR4 pathway. As, until then, no studies examined TREM-1 in the kidneys, we first characterized TREM-1 expression in human and murine renal tissues. TREM-1 was detected on tubulointerstitial cells in renal biopsies from patients with hydronephrosis, but not in biopsies from renal transplant patients with stable graft. Similarly TREM-1- and DAP12-mRNA$^+$ interstitial cells were identified by *in situ* hybridization in murine kidneys solely after ureteric ligation. To study the contribution of TREM-1/DAP12 in the evolution of CKD, we compared wild-type, TREM-1/-3 KO, and DAP12 KO mice subjected to UUO. In mice, the Trem1 and Trem3 genes are adjacent and highly homologous genes. The two receptors have similar cellular distribution, act as amplifiers of inflammatory signaling and associate with DAP12, suggesting that these two

proteins have redundant functions in the mouse. In humans, Trem3 is a pseudogene; thus TREM-1/-3 double KO better mimics the effect of blocking TREM-1 in humans.[82, 83]
During late stage of UUO (day 7 and 14), renal MCP-1 and KC levels, and macrophage influx were diminished in DAP12-deficient mice as compared to wild-type littermates. In absence of TREM-1, instead, solely MCP-1 levels were reduced at day 14. TLR4 was equally induced in all mice groups following UUO, while TLR2 upregulation was lower at day 7 in TREM-1/-3 and DAP12 KO mice. The evaluation of the fibrotic parameters showed no contribution of TREM-1 and DAP12 in collagen deposition or myofibroblast accumulation. These data indicate that intact DAP12 contributes to an inflammatory renal milieu in the advanced stages of nephropathy, in parallel with the appearance of a prominent leukocyte (macrophages and neutrophils) infiltrate. The reduced levels of MCP-1 in both DAP12 and TREM-1/-3 KO mice may indicate that this chemokine is induced partially via TREM-1/DAP12 pathway. Other DAP12-related receptors, including TREM-2, were found upregulated in the injured kidneys of wild-type animals implying that their signaling is also blocked in absence of DAP12. The DAP12 pathway was previously found to be required for proper macrophage recruitment into lungs, chemotaxis of macrophages towards MCP-1 and macrophage migration after wounding scratch assay. In addition, reconstitution experiments with DAP12-deficient macrophages showed that the association of TREM-2 with DAP12 was sufficient to restore migration.[84]
Since TREM-1 and DAP12 were found expressed only by interstitial cells and not by other renal cell types, it is reasonable to assume that the differences observed among the genotype-groups are mainly due to lack of TREM-1/DAP12 on these tubulointerstitial, presumably inflammatory, cells. It is, therefore, surprising that both knockout mice strains displayed a small but significant increase in the degree of tubular damage and edema compared to wild-type mice early (day 1 and 3) after ligation. Indeed, at the same time-points, the renal recruitment of leukocytes was minimal. Analogously, at day 1 of UUO tubular injury in TLR4 KO mice was clearly more severe than in wild-type littermates. It remain difficult to explain how TREM-1/DAP12 and TLR4 pathways can synergize to maintain tubular integrity.
In conclusion, TREM-1 appears not to be a major determinant of the magnitude of inflammation during obstructive nephropathy, possibly because its function is overwhelmed by the activation of TLRs, such as TLR2 and TLR4, which are also expressed on tubular cells besides infiltrating inflammatory cells. The work of Campanholle *et al.*, which was published almost simultaneously with ours, support the data of **chapter 4**. The authors reported TREM-1 upregulation after UUO as well as after renal ischemia/reperfusion and identified TREM-1 positive cells as M1 type macrophages. To block TREM-1 activation, a fusion protein with the mouse TREM-1 ectodomain and the human IgG1 Fc domain was used as decoy receptor. Daily treatment with this soluble TREM1-Fc fusion protein, after UUO or unilateral ischemia/reperfusion, appeared ineffective in preventing macrophage activation, injury and fibrosis in the damaged kidneys. The authors further conclude that TREM-1 is not a major target for endogenous renal DAMPs, since neither activation nor blockage of TREM-1 on macrophages altered IL-1β expression induced by DAMPs extracted from day 5 UUO kidneys.[85]

CD44s and CD44v3 in chronic renal injury and fibrosis

In chapter 2, we studied CD44 in the settings of acute inflammation and related renal injury finding a better outcome in mice lacking CD44. During chronic renal disease, mice deficient in CD44 appeared protected against tubulointerstitial fibrosis development,[20] which ultimately leads to loss of renal parenchyma and end-stage renal disease.[58] However, in contrast to the LPS- or ischemia/reperfusion-induced AKI,[19] tubular injury was aggravated in CD44-null mice subjected to obstruction of the ureter. From the studies presented in **chapters 5** and **6**, now we know that the protection of tubular cells from extensive damage and apoptosis was elicited by the CD44v3-v10 isoform, whereas the promotion of renal scarring was mediated by the standard CD44 isoform; even though other variants might be involved. By means of distinct genetically modified mice in chapters **5** and **6**, we intended to discover the functions (yet unknown) of CD44s and CD44v3 in the pathogenesis of renal injury and fibrosis induced by UUO.

CD44 expression is generally absent in healthy kidneys, but it is rapidly induced after renal damage, especially at the basolateral membrane of proximal tubular cells.[17-20]

In **chapter 5**, we used new transgenic mice, generated on a wild-type background, that constitutively overexpress either CD44s or CD44v3 specifically on proximal tubules. In order to target proximal tubular cells and to avoid potential renal development malformations, transgene expression was set under the control of the 5' regulatory promoter region of the γ-glutamyl transpeptidase type-1 (γGT-1) gene. The γGT-1 protein is exclusively expressed at high level by proximal TEC in the kidney and the γGT-1 gene is mainly expressed from 3 weeks after birth.[86]

Initial experiments with primary TEC derived from wild-type and CD44s/CD44v3 transgenic mice showed a different modulation of cell susceptibility to TGF-β1 and HGF by CD44s or CD44v3 tubular expression. In overall, CD44s overexpressing TEC and CD44v3$^+$ TEC better responded to TGF-β1 and HGF, respectively. HGF and TGF-β1 are well known for their antagonizing activities in CKD with HGF promoting regeneration and counteracting fibrotic stimuli, and TGF-β1 triggering the scarring process.[87] *In vivo*, the effects of CD44s or CD44v3 overexpression by proximal tubules became apparent after one day of ureteral obstruction. At day 1, kidneys from CD44v3 transgenic mice displayed less myofibroblasts, a trend towards decreased TGF-β1 signaling, and increased BMP-7 synthesis/signaling compared with CD44s transgenic mice. Renal HGF expression was upregulated one day after UUO in wild-type and CD44s kidneys, but not in CD44v3 kidneys. This, however, did not result in a lower rate of c-Met activation in kidneys with CD44v3$^+$ TEC. As HGF binds CD44v3,[88] we may assume that less HGF is needed to activate c-Met in CD44v3$^+$ tubules. The increased production of BMP-7 in CD44v3$^+$ kidneys is interesting as this molecule has the potential to inhibit TGF-β/Smad signaling and suppress cytokine expression in proximal TEC.[89, 90] Similarly to HGF, *in vitro* BMP-7 induced greater effects in primary CD44v3$^+$ TEC than in CD44s$^+$ TEC; the upregulation of the BMP-7 target ID-3 and the suppression of MCP-1 expression were more pronounced in BMP-7-stimulated CD44v3$^+$ tubular cells. The cytoplasmic domain of CD44 was proven to bind the intracellular BMP-7 signaling molecule Smad-1,[91] and the degree of CD44 expression on chondrocytes was shown to dictate cell

sensibility to BMP-7.[92] In addition, another study demonstrated that the binding of BMP-7 to cell surface heparan sulfates is required for BMP-7 signaling.[93] It remains an open question whether CD44v3 is directly implicated in BMP-7 binding and signaling.

Following one day of obstruction, mice with CD44s$^+$ TEC presented more tubular damage and interstitial edema. The latter could be a consequence of poorer expression of claudin-2, a tight-junction-associated adhesion molecule typical of proximal tubules.[94]

At later time-points no differences were found among the mice strains in the degree of tubular damage or CD44 positive tubules. As the transgenes are overexpressed solely in proximal TEC on a wild-type background, the renal injury-induced upregulation of the "wild-type" CD44 gene might overwhelm the transgene functions.

After evaluating the expression of the CD44 ligands HA and osteopontin,[95, 96] together with the extent of inflammatory infiltrate (macrophages, T lymphocytes), we concluded that TEC overexpression of specific CD44 isoforms had no influence on the degree of CD44 ligand accumulation nor on the inflammatory cell influx in day 1 UUO kidneys.

In the study described in **chapter 6**, we used wild-type and transgenic mice expressing solely CD44s or CD44v3 on a CD44 knockout background, and subjected them to ligation of the ureter. In physiological conditions, these transgenic mice presented a normal phenotype and absence of CD44 expression in the kidney cortex, as in wild-type mice.

During obstructive nephropathy, the expression of solely CD44v3 protected kidneys from tubular damage and fibrosis formation. In contrast, the presence of CD44s led to increased tubular damage and renal fibrosis. The diverse impact of CD44s/CD44v3 on TEC proliferation/apoptosis was proven by the high proliferative index of CD44v3$^+$ TEC even at late stage of UUO and the exacerbate apoptosis rate in CD44s$^+$ TEC. The attenuated tubular damage and the mild fibrosis seen in CD44v3 knockin mice can be attributed to relatively higher HGF production and signaling activation in the advanced stage of nephropathy. Conversely, CD44s expression associated with enhanced TGF-β1 down-stream signaling and TGF-β type I receptor expression. *In vitro* stimulation of immorto tubular cells and mouse embryonic fibroblasts with, respectively, HGF and TGF-β1 confirmed the *in vivo* observations and the results described in **chapter 5**. Both studies show that CD44s and CD44v3 inversely modulate the HGF- and TGF-β1-induced intracellular pathways and the consequential cellular responses to these growth factors. Especially the data of **chapter 6** underlie the importance of CD44v3 as beneficial co-factors during renal injury. By binding HGF through its heparan sulfate moieties and promoting signal transduction through the c-Met receptor, CD44v3 appears to endorse the renoprotective activities of HGF as inducer of cell proliferation and as inhibitor of both TGF-β/Smad signaling and TGF-β receptor expression.[87, 88, 97-100] Importantly, CD44 is implicated in the activation of latent TGF-β, through recruitment of MMP-9 to the cell surface,[101, 102] and in the TGF-β type I receptor-mediated signaling upon binding HA.[103] Although all isoforms contain the HA-binding site at the N-terminal domain, the affinity for HA can be altered by alternative-splicing and post-translational modifications such as addition of heparan sulfate, keratin sulfate and chondroitin sulfate.[104-106] In addition, inclusion of exons

v8-v10 consequences in O-linked glycosylation that reduces CD44 binding to hyaluronic acid.[107] The lower HA-binding affinity of CD44v3 in respect to CD44s[104, 107] might result in decreased TGF-β1 receptor activation and signal transduction. The higher rate of Smad-pathway activation in the presence of CD44s coincides with the results of Mima *et al.*, who reported that high expression of CD44s associates with high percentage of phosphorylated-Smad-2 positive nuclei in hepatocellular carcinoma cells. The authors showed that CD44s expression is induced by TGF-β1 hepatocellular carcinoma cells and it regulates the TGF-β–mediated cellular acquisition of a mesenchymal phenotype suggesting that CD44s plays a downstream role in TGFβ-mediated signaling.[108] The positive association between TGF-β signaling and CD44s expression is likely to be responsible for the renal fibrosis and TEC apoptosis observed in CD44s knockin mice after UUO. Another mechanism by which CD44s might trigger apoptosis is through interaction with Fas as described by Mielgo and co-authors.[109, 110]

In contrast with the previous study (chapter 5), CD44v3 knockin mice surprisingly had more myofibroblasts in the injured kidneys, which may derive from different cell-types under TGF-β1 actions.[111] Given that HGF can counteract TGF-β1-induced EMT[112, 113] and that CD44v3$^+$ TEC displayed a high expression of phosphorylated c-Met, it is unlikely that these myofibroblasts originate from transdifferentiated TEC. In support of this hypothesis, Brown *et al.* showed that an isoform switch from CD44v to CD44s is required in order for cells to undergo EMT.[114] Most probably, CD44v3$^+$ fibroblasts benefit from the proliferative effects induced by heparin binding growth factors, such as fibroblast growth factor-2 (FGF-2).[115, 116] Treatment of MEF with TGF-β1 induced a mild production of type I collagen in presence of CD44v3, suggesting that also *in vivo* CD44v3$^+$ fibroblasts are less active in producing collagens.

As in other renal diseases,[17, 19, 117, 118] CD44 was induced in injured obstructed kidneys; surprisingly, the increase in renal CD44 expression was weaker in CD44s knockin mice compared to the other strains. The pattern of CD44 expression correlated with the accumulation of β-catenin in the kidneys, which was lower in the kidneys expressing only CD44s. CD44 is a major target of β-catenin/T-cell factor-mediated transcription.[119, 120] After obstructive injury β-catenin predominantly accumulates in renal tubules,[121] where CD44 is also abundantly upregulated. Wnt/β-catenin signaling is activated by wounding and participates in the healing process.[122] Indeed, AKI triggers upregulation of Wnt proteins, which in turn contribute to tubular regeneration.[123-125] Despite its indispensable role in repair, sustained activation of Wnt-pathway can be deleterious in CKD contributing to TGF-mediated fibrosis.[123, 126] Studies demonstrated Wnt/β-catenin activation in CKD and showed that systemic delivery of the Wnt inhibitor Dickkopf-1 (DKK-1) substantially reduced fibrosis, α-SMA expression, capillary rarefaction and inflammation during UUO, but greatly inhibited epithelial cell proliferation. These effects were mainly caused by DKK-1-mediated inhibition of TGF-β-, PDGF-, CTGF-activated-MAPK and JNK signaling cascades, and largely independent of β-catenin signaling.[126]

In our model, β-catenin expression in CD44v3$^+$ tubules did not correlate with fibrosis formation highlighting the importance of other factors, such as HGF that induces β-catenin accumulation[127]

and CD44 expression[128] but counteracts fibrosis.[96] In agreement, a recent report establishes that tubule-specific knockout of β-catenin does not affect the severity of renal fibrosis or the size of renal myofibroblast population after obstructive injury.[129] *In vitro*, CD44v3$^+$ TEC stimulated with Wnt3a displayed higher levels of β-catenin and phosphorylated Akt in respect to CD44s$^+$ TEC, sustaining the relative higher β-catenin tubular expression detected in CD44v3$^+$ obstructed kidneys. Conclusively, the results of this study indicate that CD44s and CD44v3 differentially modulate regenerative and fibrotic stimuli.

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